

When Collaboration can be Dangerous

EUROPEAN nations last week made yet another attempt to keep the European Space Research Organization (ESRO) in being, this time under the shadow of a declaration by the government of France that it would leave ESRO for good if an acceptable programme is not devised by the end of the year. By all accounts, the meeting last week went as well as could be expected in the circumstances. It was the first formal opportunity for the national delegations to comment on the proposals put forward last November by Professor G. Puppi. In the circumstances it was more an opportunity for identifying the points at which compromises will be needed than an occasion for formal and final agreement. The next step will be another meeting in Holland on July 13, and although it is exceedingly improbable that substantial agreements can be made at a two-day meeting, it should by then be clear which way the wind is blowing.

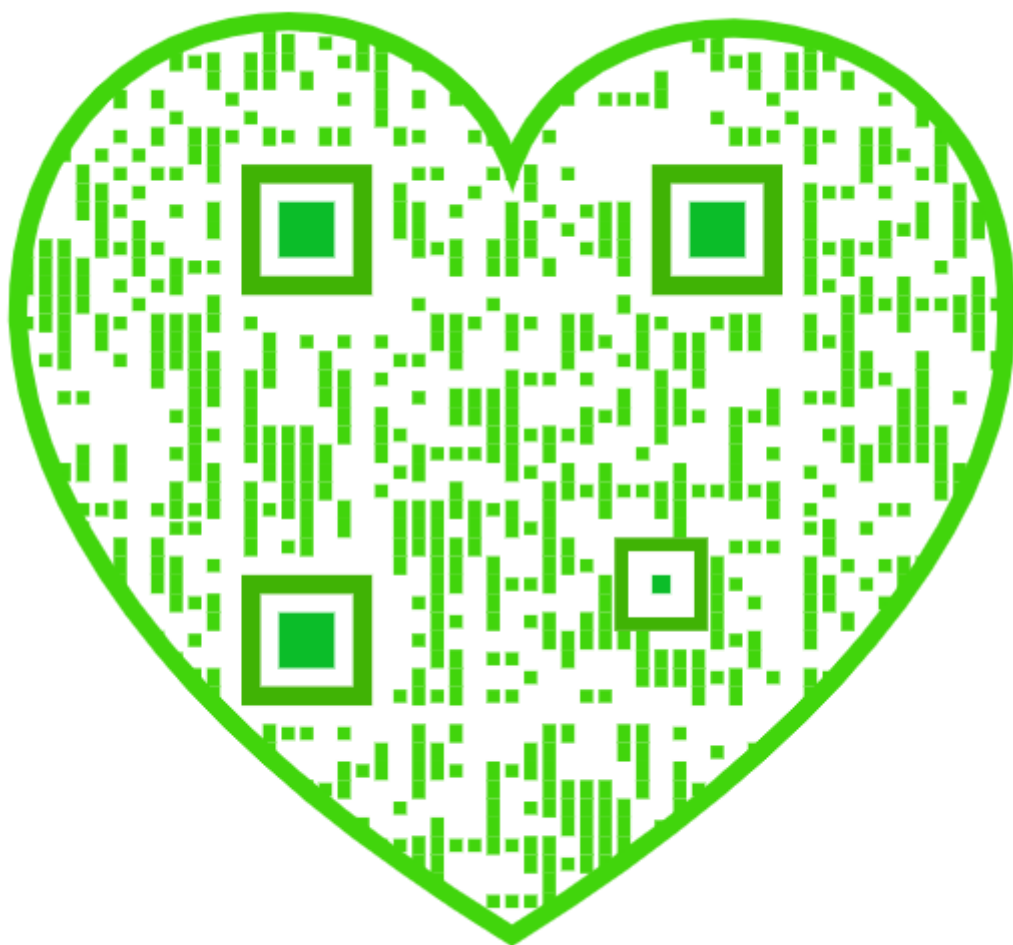
Unhappily, there are few signs at present that the negotiations on ESRO will yield much of a framework for a sensible strategy on European space research in the decades ahead. The issues which have divided the members of ESRO in the past few years seem at once too sharp and too inaccessible to rationality for compromise to be easy. The overriding question which nobody seems to answer or even ask is what space research on a European basis may be expected to provide. In many ways, the scientific programme originally conceived of as providing bread and butter for ESRO has fallen foul of the forces that brought Euratom to a standstill nearly a decade ago. Member countries are fonder of their national programmes for space research than of the combined operation. Indeed, with ingenuity, Britain, France and Germany in their different ways have been able to sustain substantial programmes of space research outside the ESRO framework, sometimes with the help of the National Aeronautics and Space Administration of the United States and sometimes, as in the case of France, with help from the Soviet Union. Given the apparently boundless ambition of governments to see the indigenous development of satellite launchers, it is understandable, if reprehensible, that ESRO has frequently taken second place in national affections, though it is also fair to say that ESRO would now have more friends among working scientists if somehow, in its early days, it had been more efficient.

If the sticky end to which Euratom has come is a warning of what may happen to ESRO, it is also luckily an indication of how ESRO might eventually escape from trouble. On the strictly scientific side, after all, what Europe needs is not so much a home-made collaborative effort in space research as some means of making sure that the partners in the enterprise are properly informed about each other's plans. In an ideal world, it would be best where ESRO's strictly scientific ambitions are concerned if member nations were to aim at creating not so much an organization for carrying out research, but as a research council able to operate on a European basis in much the way in which bodies like the Science Research Council operate nationally in Britain. In other words, ESRO should

aim to support research of various kinds in all member countries, taking money from some common pot and thus making possible not merely the design and development of satellites in countries such as Britain, France and Germany but also more down-to-earth activities elsewhere, such as the better understanding of the ionosphere, the collecting and cataloguing of information (still sorely neglected in the madcap race for information) and even conventional observations with familiar instruments such as balloons and telescopes. In spite of the revival in ESRO's fortunes since Professor Pierre Auger was succeeded by Professor Herman Bondi, it must be openly acknowledged that one of ESRO's greatest failings so far is that it has not become a focus for European understanding of space. Is it necessarily too late to win back that lost ground?

Another serious misconception within ESRO (not to mention ELDO) concerns the feasibility of winning benefits worth having from the application of space research. This is where opinion seems at present to be most seriously divided, and why the prospects of agreement may be considerably increased now that the next meeting of the ESRO council has been postponed to the point at which agreement on the enlargement of the EEC is likely to have been reached in principle but not ratified by member governments. In that euphoric period, there may easily be pressures from all the countries concerned to sign some nonsensical dotted line for the sake of European unity. The truth is, however, that the only application of European satellites that can make sense in the years immediately ahead is the development of a satellite for telecommunications. Moreover, it is clear that there can be no immediate prospect of setting up a satellite communications system comparable in technical complexity with the Intelsat IV satellites now coming into service. The best that ESRO and ELDO acting in concert could do would be to keep in being a programme of research and development that would provide a method of keeping international telecommunications by satellite in being if, by some disaster, the whole apparatus of Intelsat should collapse. As luck will have it, however, the Intelsat treaties will probably be renewed on the basis of the agreement reached last week in Washington (see *Nature*, 231, 205; 1971) and which provides an opportunity for breaking the monopoly of Comsat in the management of Intelsat some time in the next seven years. Even if chauvinism is the only motive for the separatist tendencies among European nations, is it not now more sensible that ESRO, ELDO, some marriage between them or some offspring from them, should be nurtured in the years ahead as a potential contender for the next management contract in Intelstat, than that money should be spent on the quite separate development of a European telecommunications satellite? Not merely would such a course be more prudent; it would be cheaper as well.

How is such a situation to be reached? Dispassionate observers must acknowledge that present arrangements for space research in Europe are a kind of pantomime. The European launcher development organization,



ELDO in familiar language, has been dedicated to the development of a fruitless rocket launching system based on the ex-military British rocket called *Bluestreak* but, as it happens, Britain is no longer a member. Last week, at Paris and for the past year, British delegations to ESRO have been proclaiming that there is no shortage of rocket launching capacity in Europe that could not be cured instantly by a suitable agreement with "the Americans". After several years of behaving as if it were possible for individual nations in Europe to manufacture whole rocket launching systems for themselves, the French government has swung round to the view that the only cause for which Europe could decently unite in space research is for the development of an independent tele-

communications system. The French view is so intransigent that it even suggests that the scientific programme of ESRO should be regarded as an optional extra—*à la carte* is how the negotiators put it. It will be a great waste of public money in sending all the national delegations to Holland on July 13 if it is not plainly recognized before then that the inconsistencies at present embodied in ESRO and ELDO are not merely nonsenses but signs that when faced with the need to make decisions that are unpalatable to some of them, European nations still respond by keeping in being organizations that should be disbanded. In the long run, that is as much a disservice to the European ideal as would be the outright abandonment of the notion of collaboration.

Nothing Much to Report on Transplants

ALMOST two years have gone since the Department of Health's committee under Sir Hector MacLennan argued that the British Government should take more active steps to regulate but at the same time to encourage the use of human organs for transplant operations, yet nothing much has happened. In the circumstances, Mr Tam Dalyell, the Labour MP, and his associates are to be congratulated for having set aside some of the parliamentary time at their disposal for a forlorn attempt to introduce a bill that would require the government to organize a scheme for registering the names of people willing to have their organs used for transplants at death and then to drum up support for such a scheme. To be sure, nobody expects that the bill will ever be heard of again—without active support from the government, parliamentary procedure is almost certain to cause such bills to sink without trace. Yet this is one set of circumstances in which inactivity of the kind on which the present government seems to specialize, sometimes with good effect, is misapplied.

The MacLennan Report was a sensible document. For one thing, it pointed to strictly legal imprecisions in the legislation which at present regulates the use of transplants in Britain—the Human Tissues Act (1961). What precisely is to be understood by terms such as the "person lawfully in possession" of a body only recently certified as dead, for example? Is it the hospital, or the doctor in charge, or the next of kin? Legal housekeeping alone requires that these imprecisions, comparatively unimportant where proposals for corneal grafting are concerned, should be quickly tidied up for the sake of those who work in newer fields. The committee also argued for a more explicit acceptance of seemly procedures in the conduct of transplant operations. Thus it wanted certification of death to be the responsibility of doctors independent of the transplant teams, caution in the use of live donors and a certain amount of restraint (that would not exclude the taking of blood samples) in determining the suitability of a potential organ transplant. All these are sensible suggestions which have not yet been blessed even with formal applause by the British Government.

The failure of the Department of Health to help with the setting up of what is popularly known as an organ bank is, however, more serious. The law may mend itself in the course of time, but no amount of legislation can

ensure that the supply of organs will be sufficient to meet what is bound to be a growing need. Kidney and liver transplants have become acceptable parts of surgical procedure, and would be more widely used if more hospitals were staffed and equipped to use these techniques. Although there is some evidence that donors and their families are now more ready to cooperate with surgeons, however, it is both unnecessary and unwise of the government to behave as if relations between donors and recipients could indefinitely be subject to hazards such as those of failing to find the next of kin in time or the simple difficulty of broaching the transplant issue with relatives who are plainly over-distressed by death or the prospect of it. In circumstances like these, there is every reason why a simple register of people's wishes about the disposal of their organs after death should be established and maintained on a national basis. Evidently the surgeons would prefer a system in which people's organs could be used at death without further reference to next of kin unless they had previously contracted out. Such a system works well in Sweden, for example, but there is much to be said in comparatively illogical Britain for a more modest beginning with a national register of those who would allow their organs to be so used. What harm could such a system cause? And would it not be easily accommodated alongside the putative Tissue Typing Service which the Department of Health has been struggling to create for the past three years? By its neglect of these important matters, the British Government is shrugging off its responsibilities for public health.

What can be done by outsiders to change these neglectful ways? The learned societies could complain more vigorously than they have done so far of the need for some kind of register. British medicine is not, after all, short of influential pressure groups, and even if the British Medical Association is plainly unwilling to burn at the stake for issues other than doctors' pay, the royal colleges would make useful champions of this honest cause. There is also a great deal that could be done by individual hospitals. Why not, for example, ask new patients whether they would agree to the use of their organs for transplants if they should die? These are ways in which individual hospitals and hospital management boards could do a great deal to force the inactive hand of the central government.

No Liberation for Harvard Women

UNIVERSITIES are rightly proud of the part which they have played in recent years in the advancement of all kinds of liberal causes, such as civil rights for coloured people in the United States, which is why it is ironical that universities should be among the most flagrant exploiters of women in employment. The fact that Harvard University should be the first to make a public confession of its wrongdoing by publishing last month the report of its Committee on the Status of Women in the Faculty of Arts and Sciences is not necessarily a sign that it has more to confess than other universities of similar repute and size; honesty may be a sufficient explanation. But the report does provide a good deal of evidence that the best universities are able, because of the competition which there tends to be for places on the faculty, to keep women teachers in greater humiliation than lesser institutions. The Committee on the Status of Women points out that in 1970, only two out of more than 450 tenured professors were women, that only eleven out of 222 associate and associated professors were women, but that the ranks of the untenured were well populated by women. On the basis of the statistics available, the report infers that "the situation at Harvard is only slightly worse than that at other major universities, and even Berkely is only able to boast that two per cent of its full professors were women in 1970.

The most serious abuse at Harvard, according to the Committee on the Status of Women, lies in the concentration of such women as find jobs at the university in untenured appointments as lecturers, research associates or research fellows. What happens is that many of the women concerned are unable to leave the area, possibly because of marriage, or are unable to work full-time, as during child-rearing. The university—which in this sense is to be interpreted as the heads of the departments, not simply the administration on which all calumnies are heaped—can snap up academic labour cheaply. But it is well known that untenured posts at universities such as Harvard are regarded by professional academics as stepping stones to tenured appointments either at Harvard or at other universities. People who languish for more than a few years as research fellows are likely to stay that way, and this indeed is what seems to have happened at Harvard. Women in untenured posts have no voice in the university's affairs and little hope of professional advancement.

Old-fashioned prejudice has helped to strengthen this sense of purdah. The report now published has some scarifying tales to tell of the questions which heads of departments, male no doubt, have asked of women applicants for jobs. Do you plan to have children? Will you look after your children yourself? Does your husband intend to stay here or to find a job elsewhere? The report quite properly points out that the assumptions that underlie these questions are unworthy of a university such as Harvard. It also says, without quoting chapter and verse, that many appointments of distinguished women have in the past been blocked by assumptions, implicit or even explicit, that single women academics are in some sense peculiar, that married women academics are merely appendages to the husbands whose careers must come first, that the possibility of children makes all

women poor risks and that women, in any case, cannot work full-time.

What is to be done? The particular recommendations of the Committee on the Status of Women are a mixed bag. The committee offers as a goal that the "Harvard Faculty should strive to achieve" a percentage of tenure among women equal to the percentage of women in those collecting PhD degrees at Harvard ten years ago (which is close on ten per cent) and a percentage of women in non-tenure jobs equal to the percentage of women in the cohorts of PhDs at present graduating. As a basis for what may turn out to be a negotiation, this is sensible enough. It would not, for example, cause too much upheaval in the immediate future, yet it would provide such an influential representation for women in the university's affairs that imbalances elsewhere in the system would be corrected in the course of time. The objection, of course, is that such a numerical goal is bound in some sense to be artificial. And if, as is probable, academic life is less prejudiced against women than the still more iconoclast professions of commerce, politics and the law, is there not at least a chance that women would win more than a strictly proportionate share of faculty positions if they were genuinely in favour of a complete four year tenure post on the basis of their scholarship? It would, in other words, be more equitable and in the long run more sensible if the university were to enjoin its faculty to deal fairly with all appointments, to lay down the rules to govern the filling of posts and then to let the devil take the hindmost, whatever his (or her) sex.

100 Years Ago



NOTES

It will be welcome news to astronomers throughout the world to hear that the Board of Visitors of the Royal Observatory have determined to ask the Government to grant the sum of 5,000*l.* to enable *photographic* observations to be made of the approaching Transit of Venus. It is a matter of wonder that now, when the labours of De la Rue and Rutherford have brought this most perfect means of astronomical record to a pitch of perfection which it is scarcely needful to surpass, it is still ignored in official observatories. In the matter of the Transit of Venus, it will more than double the chances of success, and Mr. Rutherford has shown that in other inquiries it enables an only moderately skilled person to do in a month what a Bessel would require years to compass by the old method. There is no doubt that the appeal to Government will be successful. Would that we had a Physical Observatory and a Board of Visitors to look after other phenomena which we are now neglecting, the observation of which is even of more importance in the present state of science than that of any number of Transits of Venus!

From *Nature*, 4, 107, June 8, 1871

OLD WORLD

NEUTRON GENERATORS

Decision Imminent

FOR the third time in nine years, the British government is to be asked to build a 100 megawatt nuclear research reactor. This time the proposal comes from the Science Research Council, which is prepared to stand the entire cost from its own annual budget. The function of the reactor would be to provide beams of neutrons for research. Neutron diffraction crystallography, for example, can be used to analyse the structure of complex molecules with a precision unattainable by other methods. The merit of a neutron generating reactor could be not only in the energy attained by the particles but also in the way in which the neutrons can be controlled. The special value of the projected British machine lies in the neutron flux density of about 10^{15} neutrons per square centimetre per second, which it should be able to generate. This is three orders of magnitude greater than the earliest reactors.

Although the new reactor may take up to eight years to complete, it is unlikely that it will be obsolete before completed, for the projected flux density represents the limit that can be achieved with present technology. Significantly, in the United States, plans for a still larger reactor have been abandoned in the recent crop of research economies.

The know-how to build a large research reactor has been available for ten years, and the problems to which the machine could be applied have been spelt out frequently by the Science Research Council. Why, then, has the project been dormant since 1962? The first proposal fell foul of the EEC negotiations in the early 1960s. When Britain's application for entry was withdrawn, it signalled the end of possible British cooperation in the construction of an £11 million Franco-German reactor at Grenoble. The Grenoble reactor, smaller and less versatile than the projected British machine, should go critical next year. The next stumbling block was the dwindling status of the UK Atomic Energy Authority (which had assumed financial responsibility for building a British reactor) in the eyes of the government. Even a deal through which the SRC would bear some part of the cost met no response from successive ministers of technology.

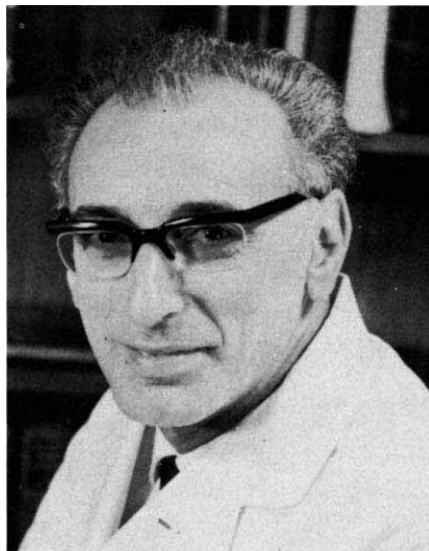
Will the latest proposal meet with more success? The SRC Committee for Nuclear Beam Research, under Professor E. W. J. Mitchell of Reading University, completed its arithmetic early this year. The reactor should cost £22 million spread over eight years, in-

cluding annual running costs estimated at £3 million. The first step will be a £2 million design study to be carried out by Harwell, which will also build the reactor. Hopes are high that if government assent is given, a start will be made this autumn.

NIMR

Medawar's Successor

THE new director of the National Institute for Medical Research is A. V. S. Burgen, FRS, Sheild Professor of Pharmacology and head of the Medical Research Council's Molecular Pharmacology Unit at the University of Cambridge. Professor Burgen, who takes up his appointment in September, succeeds Sir Peter Medawar, the immunologist, who has been in charge of the NIMR since 1962. A stroke two years ago left Sir Peter in indifferent health, and he has given up the directorship to concentrate on his research at the Clinical Research Centre, Harrow.



Professor A. V. S. Burgen

Professor Burgen will have in his care the largest MRC-sponsored medical research establishment in Britain. In 1960-70, grants to the NIMR totalled £2,355,582 against £19,135,797 for all MRC establishments. Burgen's own unit will follow him to Mill Hill next year to form the nucleus of a new division of molecular pharmacology; at present sixteen strong, the unit has been looking at the application of nuclear magnetic resonance studies to the interaction of drugs with cell membranes.

Professor Burgen said last week that he had no immediate plans for changes in the organization of the NIMR; his first task will be to learn how to run such a large and complex establishment. Burgen is, however, no stranger to major administration; for five years he was deputy director of the University Clinic at Montreal General Hospital.

RADIO ASTRONOMY

New Bodar Telescope

by our Soviet Correspondent

PLANS for the new radio telescope of the Siberian Institute of Terrestrial Magnetism, the Ionosphere and Radio-Wave Propagation have now reached the stage of "pilot" tests.

The new telescope, which is being built under the direction of A. A. Pistol'kors, Corresponding Member of the Soviet Academy of Sciences, with the close cooperation of the observatories and relevant institutes of the academy, will be a multi-element cruciform radio interferometer, with lines of antennae orientated N-S and E-W. It is to be used for solar observations in the centimetre wave-band, as part of the institute's programme of solar observations in relation to problems of long-range communications and weather forecasting. The resolving power will be some 20 seconds of arc. Tracking and data processing will be automatic, building up a complete radio picture of the Sun every few minutes.

The Bodar site will be used for observations only, data being relayed by teletype for processing in the institute's laboratories, where it will be compared with the optical observations from the other observatories. It is expected that the telescope will facilitate the recording of local sources of solar radiation, the location of solar bursts and the real-time tracking of high-speed processes in the lower layers of the corona.

One long-term objective is the mapping of the magnetic field of the Sun.

It is hoped that the "pilot" tests will be completed during 1971, and that work will go ahead immediately afterwards. Site preparation is already complete.

HIGHER EDUCATION

CNAA Looks Ahead

THE Council for National Academic Awards seems steadily to be winning public confidence as the source of degrees and diplomas for institutions of higher education other than universities and colleges of education in Britain. The annual report of the council for 1970, published this week, explains that there are now close on 24,000 students enrolled in first degree courses, more than half of them on sandwich courses. Predictably, perhaps, courses in the arts and social sciences now account for nearly a third of all enrolments. There are 869 students following postgraduate courses leading to the MPhil and PhD degrees.

NEW WORLD

FDA Called Unhealthy for Science and Scientists

by our Washington Correspondent

MAYBE the Food and Drug Administration is not one of the most disorganized, ineffective and negligent bureaucracies in Washington, but, if not, it must be the most maligned. In the last 15 years some 14 separate reports, prepared inside and outside the government, have criticized the agency's various management deficiencies. The cyclamates episode of 1969, combined with a visitation from a Ralph Nader study group which reported to the public last year, left in shreds what remained to the agency of a reputation.

Last week even these tattered remnants were brutally stripped away by the report of a high level committee appointed in a radical moment by Commissioner Charles C. Edwards shortly after he took over as head of the FDA. The new report, concerned only with the scientific activities of the agency, is an almost comprehensive catalogue of possible deficiencies in science management that ranges from its subordination of scientific facts to political and economic considerations, to bad morale among scientists, low productivity, antiquated equipment, skimpy record-keeping and even laboratories that are actually unsafe.

The authors of the report are five university medical scientists, headed by Roy E. Ritts, professor of microbiology at the University of Minnesota, who started their inquisition in March last year. Their amazement at what they learnt is evident on every page of the report, the flavour of which may be judged from the following passages:

● "The committee was distressed to find that Chicago and Philadelphia facilities [where the FDA has district offices] are antiquated, crowded and unsafe and that the Denver facilities are only barely adequate. These facilities are a disgrace to the agency and the committee can only express its alarm that FDA scientists are asked to work under such conditions. The microbiological facilities in the Chicago laboratory are so poorly arranged and unsanitary that it is pointless to even attempt bacterial identification in them."

● "The committee was disturbed to discover that the FDA did not have a systematized record-keeping system for its laboratory work. There was no evidence that laboratory data were being recorded in such a way as to be secure from misplacement or *alteration* [italics added], or to permit ready retrieval." The committee even deems

it necessary to spell out with leaden handed sarcasm that "well managed laboratories . . . are characterized by a rather firm adherence to certain generally accepted principles of laboratory record-keeping. These include bound notebooks . . . identification of the observer, the date of the experiment, description of the experiment itself and its components and procedures, pertinent notes and observations relating to the data, including reasons for discontinuance or changes and notes on preliminary conclusions and future plans. These are usually recorded in ink and the pages numbered and indexed and are preserved in the scientist's office. . . ." For the committee to have found Commissioner Edwards in need of instruction on such elementary points indicates that the standards of laboratory practice in the FDA must come close to transcending description.

● "The committee was dismayed to learn that the FDA does not have accurate information at any point in time of the identity and amount manufactured of all drug preparations, both ethical and proprietary, currently on the market in this country. This is a serious deficiency which compromises not only the FDA's knowledge of overall drug usage but effectively limits the value of any drug experience reporting system, since a correct estimate of incidence cannot be made under these circumstances."

● "The committee has been told that the processing of applications [from manufacturers to put new drugs on the market] is slow and erratic, that demands for data may be scientifically unjustified, that some reviewing officers make rather rigid and arbitrary decisions while others have trouble making decisions at all, and that interpretation of policies seems to vary from reviewing officer to officer."

● "The committee was surprised to learn [in its study of the Office of Scientific Evaluation] that dictating equipment is not used, that reports are often handwritten, that typing is alleged to be days or weeks behind schedule. . . . Simple inspection of the premises revealed crowded offices and desks in waiting rooms. Primitive conditions of this sort do more than pose obstacles in the day to day work of reviewing officers. Efficient, dignified professionalism cannot flourish in such an environment."

Horror stories apart, the most damaging of the Ritts committee's findings is its assertion that scientific facts are subordinated to political considerations in making regulatory decisions. Unfortunately the report dwells on this important aspect only in three brief passages, two of which are general statements and the third refers to cyclamates specifically.

"Some important regulatory decisions have been made in the past apparently without adequate scientific input, thus giving the impression that scientific considerations are less important than other factors in the decision-making process," the committee declares enigmatically in its general remarks about the agency. A few pages later is the somewhat clearer statement: "The committee is in no way persuaded that the scientific basis for regulatory decision-making should be modified by consideration of economic or political factors", and in the conclusion of the report is the specific accusation that "the recent FDA decision on cyclamates provides an illustration of a forced and hurried judgment with inadequate, premature or unconfirmed scientific input admixed with political and industry pressures".

It is perhaps the greatest weakness of the Ritts report that the committee apparently failed to investigate or document instances of this kind, because the low rank accorded to scientific evidence in the FDA's decision making process is clearly a root cause of many of the evils the committee has described.

Even this description is less than the full truth; at a press conference held last week to announce the report, Professor Ritts admitted that previous versions of the report had been harsher still. The version now made available makes sufficiently evident that the Food and Drug Administration is not adequately equipped in its science manpower or resources to protect the interests or safety of 200 million American consumers.

But even this revelation may not be an adequate stimulus to reform. In the first place, it has been made many times before, if seldom so pointedly, in the unending flood of reports that have criticized scientific research ability of the FDA since 1955. Second, the Ritts committee left no instructions as to who should enforce its recommendations for reform or how. Commissioner Edwards, in putting a best face on the report, said last week that the FDA has

already adopted and implemented many of the committee's recommendations, but in fact only 9 of the 58 recommendations are stated in the Ritts report to have been already implemented, and these are, by and large, the least important.

The FDA's publication of the report is no guarantee that the agency intends to act on it. Release of the report seems to have been prompted by enquiries from Senator Abraham Ribicoff and Representative L. H. Fountain who a few weeks ago asked to see copies. Ritts, at any rate, was taken by surprise at the sudden decision to release his committee's report. "If I had known we were going to have this hoo-ha this afternoon I'd have written it differently," he stated at last week's press conference. Edwards certainly seems to have no intention of implementing the recommendations in more than piecemeal fashion. Asked last week what the full cost of the proposed reforms might amount to, he agreed that it would be double the FDA's present budget, which is tantamount to saying they are impracticable. Moreover, the chief thrust of the committee's major set of recommendations, that scientists should be given a louder voice in the affairs of the agency, runs up against a discouraging series of precedents, the most recent of which is the apparent demise of a in-house supervisory committee of scientists appointed by Edwards shortly after he took office.

Even if the suggestions of the Ritts committee were followed in full and the agency's scientific operations organized on a proper footing, it is far from obvious that the FDA's problems would vanish overnight. Two principal sources of criticism of the FDA, Congressman Fountain's subcommittee on intergovernmental operations and Ralph Nader's Center for the Study of Responsive Law, agree that the scientific competence of the FDA is not the main issue.

A staff member of the subcommittee says that a strong enforcement posture on the part of the FDA and an evident readiness to apply the legal sanctions with which the FDA is equipped is the key to securing compliance from manufacturers. According to James S. Turner, a member of the Nader Center and author of the center's influential report on the FDA,* "The Ritts people have done a great job in showing up the scientific problems of the FDA, but have failed to grasp the context which creates those problems, which is that the FDA is not in a position to implement policy."

The long-drawn-out paralysis of the FDA is hard to understand even in historical terms, although Turner in his book, *The Chemical Feast*, traces the start of the rot to the sabotage by the Secretary of Agriculture of the first regulatory action taken under the Pure Food Act of 1906. The FDA's usual defence, that it does not have money to regulate properly the food industry with its annual turnover of some \$125,000 million, is hard to reconcile with the fact that in every year from 1960 to 1969 the agency failed to use all the money Congress appropriated for it.

It is true that frequent reorganizations and shake-ups—there have been five in as many years—have given the agency little peace, but the changes have been the effect, not the cause, of the agency's ineffectiveness. Nor can the agency legitimately argue that it lacks the legal sanctions to act against manufacturers. The legal powers with which Congress armed it have been frittered away by administrators who use their discretionary powers to avoid actions enjoined by the law. Whenever supporters of tighter regulations for the food industry seek broader authority for the FDA from Congress, the Nader study argues, the food industry lobbyists convincingly argue that the FDA is not responsibly handling the authority it already has.

The only law which does not allow administrative discretion is the notorious Delaney amendment which forbids the presence in foods of any chemical shown to cause cancer in animals. (It was this law which was invoked by Robert H. Finch, Secretary of the Department of Health, Education and Welfare, when he ordered cyclamates off the market, although the sweetener could have been banned by removing it from the list of substances generally regarded as safe (the GRAS list).)

Having failed for so long to threaten the manufacturers with such legal powers as it has, the FDA has been reduced to trying to cooperate with industry, an attitude which has only intensified the pressures which industry is prepared to bring against it. Since the FDA is known to act only in the last resort and to be susceptible to pressure because of its weak position, it is in every manufacturer's interest to try to thwart or delay a decision against his product.

The principal way in which pressure is exerted seems to be through constant meetings between industry representatives and FDA officials—including Commissioner Edwards—and unrelenting suasion by lobbyists of the few individuals in the agency who may be responsible for a particular decision. Thus or otherwise the scientific facts

on which a decision should be based are, in the words of the Ritts committee, "modified by consideration of economic or political factors". The consequence, in the words of the Nader study, is that "the various FDA food standards and exemptions now in force read like a catalogue of favors to special industrial interests".

A particular feature of the FDA's recent history which has certainly contributed in large measure to its present weakness is the subordinate and faltering role of its scientific activities. As early as 1955 the First Citizens Advisory Committee on the FDA urged that a strengthening of scientific research within the agency would "do much to upgrade the professional competence, elevate the morale of the scientific workers and contribute to the effectiveness of the FDA". The same recommendation was made by a committee of the National Research Council in 1956 and repeated by a special team of consultants which in 1958 complained: "One hundred and twenty-two professional scientists are a woefully small group with which to resolve the manifold complex problems arising in the food, drug, and cosmetic areas. In recent years the resources of the FDA have been swung from one emergency to another." Reports on the agency's scientific competence grow increasingly acerbic as the years go by.

By 1959, the scientific director of the FDA, Paul L. Day, was compelled after a 6 months' study to confess his amazement at the "scientific provincialism" prevailing in the agency. Day recommended, just as the Ritts committee has now done, and probably with the same effect, in favour of a "scientific advisory board of distinguished scientists from universities, foundations, industry and other government laboratories".

A few months after issuing his report, in October 1960, Day observed that "there are many more scientists in FDA laboratories than at any time in the past, but there is good evidence that imaginative scientific work is at a low ebb. At a time when scientific research is recognized as the open sesame to progress in any field . . . FDA's research productiveness has been, in recent years, near an all time low". Shortly afterwards Day left the FDA under a cloud—apparently because his public statements were regarded as a mark of disloyalty—but nothing was done to remedy the abuses he described. In the meantime another committee of the National Research Council called for the establishment of a scientific advisory council, an idea which finally bore fruit with the appointment, some 6 years later, of a group of part-time science advisers to improve the quality of research in FDA district offices.

* *The Chemical Feast* by James S. Turner. Grossman; New York, 1970. \$0.95.

According to the Nader study group, which was able to study the minutes of the annual meetings of these science advisers, no recommendations for improvement were ever made and the deputy commissioner of the FDA, who was most responsible for the agency's central activities, did not even know the meetings were being held.

In interviews with FDA scientists in the latter half of 1969, the Nader investigators found that the environment for doing science in the agency was not conducive to maximum creative productivity. "Several researchers interviewed," the Nader report relates, "read from 'atrocious logs' in which they kept a detailed and regular account of assaults on their scientific integrity. For example, some individuals in a supervisory capacity routinely sign their names to research work they have not conducted; in one division, invitations to attend scientific meetings were dispensed not routinely but to reward . . . 'yes men', 'pets' and 'stooges' of the supervisor. . . . The most often repeated comment from dissatisfied scientists was that the FDA constantly interferes with medium and long range projects, making it almost impossible to complete them. At least part of the reason, they assert, is that certain results might embarrass the agency either by showing a lack of scientific support for an already established regulatory policy or, conversely, establishing scientific evidence of a hazard which there is no legal authority to bring under control."

The Nader report also makes an eloquent case for freedom of publication, a point only faintly echoed by the Ritts committee: "Science is best served," the Nader study states, "when ideas and information circulate freely within the scientific community. Scientists at the FDA should be allowed to publish their findings in appropriate journals without administrative restraint, but they are not. All writings done by FDA scientists must be reviewed and approved for publication by the agency's writing review committee. As a result of this procedure, several scientists have been unable to publish important scientific discoveries; others have published their articles without agency sanction."

The most recent official review before the Ritts committee was a report compiled in 1968 under Frederick V. Malec, assistant secretary of HEW, which advised that more than any other incentive, "the opportunity for the scientist to have a voice in the conduct or kind of research in which he is involved is of primary importance to his professional status and feeling of involvement".

Edwards, who replaced Commissioner Herbert L. Ley in December

1969 after the cyclamates episode and the Panalba case had caused friction between Ley and the then Secretary of HEW, chose to appoint a new review body, the Ritts committee, rather than implement immediately the recommendations of the Malec report. The final verdict on the scientific competence of the FDA was delivered by Commissioner Ley who told the *New York Times* two weeks after leaving office that "A majority of the medical staff are retreads, people who have suffered coronaries or who have personality problems; at the moment they're the only people the FDA can get. Merely putting more money into the agency will not change it. A more highly motivated staff and better administration of it also is needed."

The Ritts report does little to contradict Ley's verdict, although its criticisms are aimed at the organization of science in the FDA rather than individuals. Indeed, the committee states explicitly that it regards the FDA scientists as competent. Nor is the Ritts report all black. Praise is given to the Division of Veterinary Research and in general terms to the district laboratories which, within their limitations, "are doing a laudable job, certainly exceeding what the general public and the scientific community are aware of and give them credit for". The committee also states that certain FDA laboratories possess advanced technology, good morale, and high productivity and are first rate by every standard.

The only occasion on which the Ritts committee singles out individuals, though not by name, is to castigate certain scientists for having "taken their incomplete or preliminary findings directly to the public". Whatever high sense of purpose may inspire these disclosures, the public is "poorly served by reports based on unconfirmed data which it is in no position to evaluate". Scientists who go to the public over their superiors' heads are "subverting" the FDA's mission, the committee declares, and the Commissioner should take steps to identify them and "correct this anomaly".

Ritts declined last week to name the scientists who were subverting the FDA's mission, but Edwards said he knew who most of them were. Although in ordinary circumstances the committee's concern to shield the public from undigested data would be well taken, the scientists who have given their results to the public have done so only under severe pressure, and in the expectation that their results would be suppressed at higher levels of the FDA bureaucracy. "The feeling is quite prevalent among scientists in district offices that the cases they have prepared with care are knocked down

by the people in Washington," a former FDA official said last week.

According to Nader Report author James Turner, "the chemists in the field work hard, but their efforts are subverted on a routine and systematic basis by the management of the agency". The Nader study describes an instance in which a recommendation by an FDA scientist that the marketing of cyclamates be stopped was deleted by a superior from his memorandum without the author's authorization or knowledge.

The best known instance of an FDA scientist taking results directly to the public was when Dr Jacqueline Verrett—still an employee of the FDA—gave a television interview on October 1 1969 about her findings that cyclamates induced birth deformities in chickens. Verrett, in fact, did not take the initiative, but was asked for the interview by the television company, which had learnt of her experiments through the scientific press. She also sought and received approval for the interview from the Deputy Commissioner of the FDA. The fact that cyclamates were banned 17 days later, for the wrong reasons and under the wrong law, thereby initiating a damagingly ludicrous sequence of reversals, counter-decisions and backtracking by the FDA's top management, may to some degree account for the agency's dislike of "subversive" scientists. But there was no need for the Ritts committee to endorse this prejudice.

The Ritts committee is only the last of a long succession of groups that criticized the scientific research efforts of the FDA. It may be overstating the case to say, as Turner said last week, that Commissioner Edwards is "sitting on a garbage pile of an agency". But the Ritts committee report can leave no doubt in the public mind that something is festering. Strengthening the agency's scientific base, as the Ritts committee recommends, and creating a constituency among consumers, as the Nader report advocates, are two obvious expedients for building counter forces to industry pressure. But the FDA's present policy, at least according to its critics, is still to cooperate with industry rather than fight it. "Edwards feels he would get further in enforcement if he could get industry to cooperate—he's only beginning to learn that cooperation sometimes makes you do things against your best interest," a staff member of the Fountain Subcommittee said last week. The extent to which Edwards implements the major recommendations of the Ritts report over the next few months will determine whether he has resolved to cleanse the Augean stables in the FDA or to let matters continue in the pattern of the past 15 years.

How to Reform the FDA

● The chief recommendations of the Ritts committee concern the setting up of a system of science advisory committees to give scientists a more powerful say in the agency's affairs and to correct the predominance given to compliance branches of the agency over the scientific branches. The committee recommends that each of the four bureaus of the FDA should be guided by a National Advisory Council including scientists versed in the relevant disciplines as well as representatives of community and consumer interests. Scientific Review Committees should be established to advise on continuing problems, such as screening applications to market new drugs, and *ad hoc* scientific advisory committees should be created to deal with particular issues. An in-house Scientific Planning Committee should be set up consisting of the heads of bureaus and the associate commissioners for science and medical affairs.

● In studying the present FDA programme of awarding research contracts, the committee found that "serious deficiencies exist at all levels of the process". Its suggested remedies—which have been accepted in principle or already effected—include such elementary advice as giving the widest possible publicity to the contract programme so that every interested scientist has an opportunity to apply, appointing full time project managers to ensure the purposes of the contract are met, and having contracts reviewed by the proposed Scientific Review Committees.

● The 17 district offices of the FDA (of which the committee visited 12) handle responsibilities that are "literally overwhelming" and can do no more than spot check the food and drugs entering the market. As a result an appreciable number of marketed drugs—25 per cent according to some studies—are substandard. The committee recommends that drug certification should be transferred from the district laboratories and centralized in a National Center for Drug Certification to be established by the FDA. Meanwhile the district offices should become more specialized, their facilities improved, and their work made more generally known in order to develop a favourable public constituency.

● Turning to the four bureaus of the FDA, the Ritts committee recommends that the Bureau of Veterinary Medicine be incorporated into the other three bureaus of the agency since the purported uniqueness of veterinary drugs does not justify its separate existence. The veterinary research division of the Bureau, for which the committee has high praise, could form the basis of a new drug research group in the Bureau of Drugs. These suggestions, which Edwards has interpreted as a "recommendation to abolish the present Bureau" have already been rejected by the FDA for reasons that are unapparent.

● The Bureau of Foods, already undergoing a major reorganization, nevertheless suffers from most of the general criticisms of science management, and indeed was the source of many of them. The only bureau laboratory studied by the Ritts committee, the National Center for Microbiological Analysis at Minneapolis, is understaffed and so badly managed that the committee failed to identify any "discernible method by which important research problems in microbiology are identified. . . . The committee concurs that NCMA is largely a series of existing job slots". The laboratory should be abolished and its functions assumed by the Office of Food Hygiene and Sanitation. The committee lists a series of projects which the laboratory should have been studying and which are presumably at present being neglected by the FDA, including such basic questions as the relationship between bacterial R factors and antibiotics in animal feeds, and the contamination of foods and drugs with viruses.

● The committee finds that the Bureau of Drugs is "not yet meeting all of [its] objectives in an ideal manner" but that there is no reason why the bureau cannot develop "in this decade" into an efficient and professional organization. The committee recommends the bureau strengthen its scientific staff, particularly in the area of statistics and epidemiology, so that its laboratories "would be in a position to confirm or extend data supplied by manufacturers and investigators". The bureau should establish a National Drug Experience Reporting System with a view to learning about the

effect of drugs on the population. The Commissioner should ask Congress for legal authority to compel the drug companies to let the FDA know what drugs they are putting on the market and in what quantities.

● The committee is particularly critical of the way in which the Bureau of Drugs handles one of its most important functions, the review of applications to put new drugs on the market. Suggested remedies are that the FDA medical officers who "all too often [have] neither research experience nor prestige among [their] scientific peers", be upgraded by receiving better training, better support from the administrative hierarchy of the agency, and the support of a pharmacologist, a chemist and a statistician in the review of new drug applications.

● In conclusion, the Ritts committee summarizes its recommendations as a demand for "better central science planning, increased participation of scientists in this planning, improved management and communication practices throughout the agency, wider use of extramural scientists on councils and committees, improved review and management procedures for grants and contracts, increasing specialization and regionalization of the district laboratory operation . . . development of improved training and management procedures for the review of protocols in the Bureau of Drugs, and a general call for an agency-wide attitude which understands that good science is the fundamental basis for effective decision-making by a consumer protection agency in our complicated technological society. . . . Finally, the committee feels that the FDA is not well understood. Long battered in public—by industry, by the medical profession, by the press and all too often by itself—the FDA is now without the broad base of public support a health-oriented consumer protection agency ought to enjoy in our society. . . . The committee believes that a valuable by-product of a more open relationship between the FDA and extramural scientists could well be a broader base of support in the scientific establishment. For similar reasons, the FDA has much to gain from a high quality, imaginative approach to consumer education".

NEWS AND VIEWS

Communication between Nerve and Muscle

THE transmission of excitation across the neuromuscular synapse reflects only one aspect of the complex relationship between nerve and muscle. Many of the normal properties of muscle are dependent on an intact supply of motor nerves, interruption of which evokes a series of morphological, physiological and biochemical changes in the muscle which are known as denervation atrophy. Features of the vertebrate skeletal muscle fibre that are known to be dependent on such "trophic" influences include the stability of membrane and intracellular constituents, the acetylcholine sensitivity of the fibre membrane, the cholinesterase activity of the muscle, the speed of contraction of the muscle, the rejection of any further innervation of the fibre, and certain enzymatic activities differentially associated with red and white muscle.

The nature of the trophic information passed from nerve to muscle is controversial. It is not, of course, necessary that the same molecular moiety be responsible for all the trophic phenomena. One viewpoint is that the normal neuromuscular transmitter, acetylcholine, is involved. Another attributes the trophic effects to an alternative, unidentified, substance, and a third group claims that the "atrophic" changes can be prevented by muscle activity, implying that the atrophy following nerve section is secondary to the resultant muscle inactivity.

The failure, in the past decade, to differentiate adequately between these opposing views on the basis of *in vivo* experiments presumably reflects the number and complexity of the variables involved. The more artificial but also, it is hoped, more controlled *in vitro* experiments may thus help to elucidate the molecular events involved in these trophic interactions. The first observation that contacts could form between tissue cultures of nerve and muscle was made more than sixty years ago, but it is only in the past few years that unequivocal evidence has been obtained that the formation of synapses may occur in tissue culture. On page 296 of this issue, Harris, Heinemann, Schubert and Tarakis report an elegant tissue culture study on the effect of contact between a neural cell and a developing muscle cell on the distribution of acetylcholine sensitivity over the muscle cell membrane.

In the normal muscle there is an area of very high sensitivity to acetylcholine in the region of the motor end-plate whereas the rest of the muscle membrane seems insensitive to acetylcholine. In denervated muscle or in developing muscle before the onset of innervation, the membrane displays a uniformly high sensitivity to acetylcholine throughout its extent. This region of high sensitivity is progressively restricted to the site of innervation on innervation or re-innervation; this restriction is thus one of the trophic effects of nerve on muscle. Harris and his colleagues decided to observe the effect on cultured rat skeletal muscle myotubes (a stage in the development from primitive myoblasts to mature striated muscle fibre) of contact by a process from cultured mouse neuroblastoma cells. The sensitivity of the membrane to acetylcholine was measured by inserting a microelectrode into the muscle cell and recording the change in membrane potential in response to very small amounts of

acetylcholine iontophoretically applied at various sites along the membrane of the muscle fibre. Harris *et al.* found that in the non-innervated fibre there was a uniform sensitivity to acetylcholine but that in those fibres contacted by a process from a neuroblastoma cell there was usually a very high sensitivity at the site of contact, associated with a considerable lowering of the sensitivity over the remainder of the fibre. Close contact between the neuroblastoma cell process and the developing muscle fibre seemed sufficient to mediate this trophic effect because the authors were unable to demonstrate that a true synapse had formed or that synaptic transmission had occurred.

Harris *et al.* are modest in their conclusions and merely comment that their system may well be a promising one by which to study the mechanisms involved in synapse formation and the trophic effects of nerve on muscle. In fact their observations already raise some intriguing questions. The trophic effect they demonstrate is exerted by the processes of neuroblastoma cells which are derived essentially not from motor neurones but from the sympathetic system, and the enzymatic composition of which is thus characteristic of sympathetic neurones. It is possible therefore that some of the trophic effects of nerve on muscle can be produced as effectively by sympathetic nerves as by motor nerves. If further studies indicate that nerve fibres which do not release acetylcholine from their endings can produce the trophic effects, this would be powerful evidence against the acetylcholine hypothesis.

Harris *et al.* also report that the more differentiated of their muscle cells spontaneously propagate action potentials of a frequency of 1 s^{-1} , and these cells also contract spontaneously. If these non-innervated but active cells were found to possess a uniformly high sensitivity to acetylcholine over their entire surface this would be damaging to the view that such sensitivity is merely a reflexion of muscle inactivity.

Most work on the nerve-muscle relationship emphasizes the dependence of muscle on nerve and it is sometimes overlooked that the relationship is reciprocal. Developing motor neurones which fail to establish contact with muscle die. Separation of a motor neurone from its muscle fibre or contact between a motor axon and denervated muscle both evoke marked changes in neuronal metabolism. The selectivity with which neurones form connexions only with the appropriate muscles during normal development argues for refined intercellular recognition mechanisms, the nature of which is unknown, of a much higher degree of specificity than those normally included in the trophic range.

The interdependence of nerve and muscle thus requires the extensive exchange of information. Harris *et al.*'s work represents a significant advance in the techniques available for investigating the language with which cells communicate. Some further light on the matter has just been provided by Robbins and Yonezawa (*Science*, **172**, 395; 1971) who describe the onset of chemical transmission in rat neuromuscular synapses formed in tissue culture.

POLYPEPTIDES

Polyproline Observed

from our Molecular Biology Correspondent

THE study of synthetic polypeptides is a gentlemanly pursuit which has been happily spared by the surging tide of molecular biology. At a remove, it nevertheless continues to provide lessons for the protein chemist, and the present state of knowledge of cooperative processes derives to no small extent from the theory of helix-coil transitions developed to explain the phenomena observed in polyamino-acids. One of the most absorbing of these has always been polyproline, partly because it provides a unique example of a conformational change arising from an isomerization of the peptide bond, and partly because it represents the most basic model for collagen.

Polyproline exists in two ordered states: form I is a right-handed helix in which the peptide bands are in the *cis*-configuration, and form II, a left-handed helix with *trans* peptide bonds. According to solvent and temperature, slow transitions can be made to occur between these two states, the mechanism of which has been a matter of conjecture. The most illuminating study on this subject comes now from Torchia and Bovey (*Macromolecules*, **4**, 246; 1971), who have used high-resolution proton magnetic resonance (220 MHz) to follow what happens when polyproline I is dissolved in water. Peaks caused by the protons on the α -carbon atoms in the *cis* and *trans* forms can be distinguished, the former being split into two components, one of them corresponding to only one proton per polymer chain of twenty-five residues. As the isomerization proceeds, the major *cis* proton resonance progressively gives place to one in the *trans* position, whereas the smaller one remains almost constant until the reaction is largely complete. The fraction of *cis* form can be determined from the areas under the peaks, and its conversion follows zero-order kinetics.

These observations are sufficient to define a mechanism for the transition: in the first place inspection of the structural formula of the repeating unit shows that a *trans* α -carbon proton will see essentially the same environment whether its neighbour on the N-terminal side is itself *cis* or *trans*. By contrast a *cis* residue on the C-terminal side brings the two adjoining α -carbons into a different relationship than when it is a *trans* residue. Thus the junction between two runs of *cis* and *trans* residues will give rise to a unique *trans* resonance from the residue at the junction, only if the *cis* block is on its C-terminal side. The converse is true for the *cis* proton signals. On the evidence of the zero-order kinetics, the single

unique *cis* proton per chain throughout the transition, and the absence of splitting in the *trans* resonances, it then seems to follow that the isomerization goes stepwise, one residue at a time, from the C-terminal end.

In addition to this pleasing syllogism, Torchia and Bovey have been able to clarify the nature of the structural collapse that has long been known to occur at high concentrations of some salts. Two explanations had previously been advanced: an increase in the range of permitted backbone torsional angles, so as to allow deviation from the geometry defining the helix, without isomerization of the *trans* peptide bonds, or the introduction of *cis* bonds at intervals. The absence of line-sharpening, which would accompany increases in freedom, and the appearance of the characteristic *cis*-resonance demonstrates that the second explanation is the correct one. The unfavourable nature of *cis-trans* junctions is thought to be overcome by the ability of *trans-cis-trans* or *trans-cis-cis* constellations of peptide carbonyls to bind cations.

Mattice and Mandelkern (*J. Amer. Chem. Soc.*, **93**, 1769; 1971) have examined the viscosity of polyproline II in water as a function of molecular weight. The intrinsic viscosity can be related to a function that characterizes the rigidity of the molecules in solu-

tion. In high concentrations of salts, such as calcium chloride, the values of the function reflect the collapse of the stiff, extended structure that Mattice and Mandelkern infer to be present in water solution, and they show, in terms of the theory relating molecular dimensions with torsional angles in the backbone, that the introduction of only 5–10 per cent of *cis* peptide bonds would be sufficient to account for the changes.

The arguments concerning the nature of the transition between forms I and II, it should be noted, relate only to aqueous solution, and it seems that a very different situation obtains in other solvents. In a series of solvent systems studied by Winklmair, Engel and Ganser (*Biopolymers*, **10**, 721; 1971), first-order kinetics are observed. An analysis of the kinetics, as observed by a relaxation method, involving perturbation of the system by changing the solvent composition (analogous to the temperature-jump method), leads to a remarkably good fit of the results. The transition in these solvent systems is strongly cooperative, with a very slow rate of nucleation (that is to say formation of *cis-trans* junctions), compared with propagation. These results constitute an interesting test of the theory of conformational transitions, developed in its most refined and general form by Schwarz.

Inhibitory Role of Catecholamines in Ganglia

IN next Wednesday's *Nature New Biology*, de Groat and Saum present new evidence for an inhibitory function of catecholamines in ganglionic transmission. The authors have studied ganglionic transmission in the parasympathetic ganglia on the surface of the cat urinary bladder, using electrophysiological methods, and have recorded the responses evoked in postganglionic fibres by stimulation of the preganglionic pelvic nerve. Simultaneous stimulation of the hypogastric nerve—the sympathetic input to the bladder—inhibited ganglionic transmission in the parasympathetic ganglion on the ipsilateral side. This inhibitory effect of sympathetic stimulation could be blocked by an alpha adrenergic blocking drug, dihydroergotamine, and the inhibitory effect of sympathetic stimulation was mimicked by close arterial injection of small amounts of adrenaline, noradrenaline or dopamine. Histochemical studies had previously revealed that catecholamine-containing nerve terminals make intimate contact with the parasympathetic ganglionic neurones (Norberg and Sjöqvist, *Pharmac. Rev.*, **18**, 743; 1966), suggesting therefore that the sympathetic input to the urinary bladder may inhibit the tissue at least in part by blocking ganglionic transmission in the excitatory

parasympathetic nervous system.

The presence of catecholamine-containing nerve terminals has also been shown in many other autonomic ganglia, of both the parasympathetic and sympathetic nervous systems. Furthermore, there have been various descriptions of the existence of small adrenergic interneurons, or chromaffin-like cells in various ganglia of both divisions of the autonomic system (Norberg and Hamberger, *Acta Physiol. Scand.*, Suppl. 238; 1964; Jacobowitz, *J. Pharmacol. Exp. Ther.*, **158**, 227; 1967). According to a recent report, such small cells in mammalian sympathetic ganglia contain the catecholamine dopamine, rather than noradrenaline which is present in the postganglionic neurones of the sympathetic system (Bjorklund *et al.*, *Acta Physiol. Scand.*, **78**, 334; 1970), suggesting that in addition to the well known role of noradrenaline at neuroeffector junctions of the sympathetic nervous system, catecholamines may also be involved as inhibitory transmitters modulating ganglionic transmission in the autonomic nervous system. Such inhibitory effects may be mediated either by nerve terminals of sympathetic postganglionic neurones, or in some cases may involve small adrenergic inhibitory interneurons within the ganglia.

MITOCHONDRIA

Where the Genes Reside

from our Cell Biology Correspondent

THE notion that mitochondria are the evolutionary descendants of some form of bacterium, or bacterial like organism, is nowadays widely accepted. The evidence for this suggestion, of course, comes from comparative biochemistry. Mitochondria have their own protein synthesizing machinery which has many of the characteristics of that of bacteria and can readily be distinguished from that of the eukaryotic cell cytoplasm in which the mitochondria reside. Furthermore, mitochondria have their own genomes or, to be more precise, circular DNA molecules. But the amount of DNA in a mitochondrion is not sufficient to specify all the proteins and RNAs involved in mitochondrial protein synthesis let alone all the enzymes and structural proteins of these organelles. Quite clearly, if mitochondria have evolved from symbiotic microorganisms, during that evolution many of the genes essential for the survival of mitochondria have come to reside in the host cell chromosomes, and the evolutionary advantage to mitochondria of an autonomous protein synthesizing apparatus becomes far from obvious.

The specific chain elongation factors required for the synthesis of proteins on the "70S" mitochondrial ribosomes are a case in point. According to Parisi and Cella (*FEBS Lett.*, **14**, 209; 1971), in the yeast *Saccharomyces cerevisiae* these enzymes, although required specifically for mitochondrial protein synthesis, are coded by nuclear DNA. For Parisi and Cella have simply shown that two strains of "petite" yeast contain elongation factors capable of catalysing protein synthesis by bacterial ribosomes even though the mitochondria of these strains are known not to support protein synthesis and their DNA is believed to be unable to specify active proteins. Moreover, they have also shown that chloramphenicol, which inhibits bacterial and mitochondrial protein synthesis, does not block the synthesis of these elongation factors on 70S ribosomes in either wild type or "petite" yeast. It seems therefore that these elongation factors, like the structural proteins of *Neurospora* mitochondrial ribosomes, are specified by nuclear genes and are also synthesized on cytoplasmic ribosomes.

By contrast, Rabbitts and Work (*ibid.*, 214) report experiments which indicate that the 18S and 12S RNA moieties of the mitochondrial ribosomes of chick liver cells are specified by mitochondrial DNA. After labelling the chick cells with ^3H -uridine, they isolated the RNA associated with mito-

chondrial fractions and resolved it into three components—28S, 18S and 12S—by gel electrophoresis. Only two of these types, the 18S and 12S RNAs, can be isolated from a preparation of mitochondrial ribosomes, which, in the conditions Rabbitts and Work used, sediment at 55S.

To decide whether the 18S and 12S RNA are specified by the mitochondrial DNA rather than by nuclear DNA, they exposed cells simultaneously to ^3H -uridine and ethidium bromide, a drug which allegedly selectively inhibits the transcription of RNA from the circular mitochondrial DNA. After such treatment, the amount of ^3H -uridine label in the mitochondrial RNAs was markedly reduced, but ethidium bromide had no effect on the incorporation of this precursor into the 28S and 18S RNAs of cytoplasmic ribosomes. Rabbitts and Work conclude therefore that the RNAs of mitochondrial ribosomes are specified by the mitochondrial DNA; it would, of

course, be interesting to see whether hybridization tests bear out this prediction.

And what is the site of synthesis of the outer and inner membranes of mitochondria? According to Neupert and Ludwig (*Europ. J. Biochem.*, **19**, 523; 1971), who have separated these two membranes of *Neurospora* mitochondria and characterized each by electron microscopy, associated enzyme activities and associated pigment, the single chief protein component of the outer membrane is made on cytoplasmic ribosomes. Its synthesis *in vivo* is susceptible to inhibition by cycloheximide. By contrast, at least some of the twenty different proteins found in the inner membrane seem to be synthesized within the mitochondrion, for their synthesis *in vivo* and *in vitro* is insensitive to this drug. But whether the genes for these proteins made on mitochondrial ribosomes reside in the mitochondrial rather than in the nuclear DNA is another and unresolved question.

Calling the False Reverse Transcriptases

SINCE the discovery of reverse transcriptase (the enzyme in tumour viruses which is capable of using the single stranded viral genome as a template for the synthesis of a double stranded DNA) was announced by Temin and Mizutani and Baltimore just a year ago, much confusion has arisen about the distribution of this enzyme. For as soon as Spiegelman reported that synthetic RNA/DNA hybrids are very efficient templates for these viral enzymes, numerous groups began to use these synthetic nucleic acids to search for reverse transcriptase activity in extracts of cancer cells and normal cells as well as tumour viruses. Polymerase activities capable of using these hybrids as templates for DNA synthesis were duly found in all these sources, but, as Todaro and his colleagues have now proved, such findings do not necessarily mean that cancer cells and normal cells contain the same enzyme as tumour viruses. Their report published in *Nature New Biology* next week is a timely antidote to rampaging oversimplification.

Their experiments are in essence simple enough. They have partially purified and compared the DNA polymerases in mouse leukaemia virus particles, untransformed mouse cells and mouse cells transformed by mouse sarcoma virus, using calf thymus DNA and synthetic DNA/RNA hybrids, poly rA.dT of different sizes, as templates. Furthermore, they have prepared antisera against the reverse transcriptase in mouse leukaemia virus particles and tested its inhibitory effects on the DNA

polymerizing enzymes purified from transformed and untransformed cells.

Mouse leukaemia virus particles have a single enzyme capable of using both these templates for DNA synthesis; this enzyme is reverse transcriptase as defined by Temin and Baltimore and it can also use the viral genome as a template. From extracts of untransformed mouse cells they purified a DNA polymerase which can use calf thymus DNA but not the synthetic hybrid as a template, and a second enzyme which can use both templates. This enzyme, however, behaves very differently from virus reverse transcriptase on chromatography. Finally, in mouse cells transformed by mouse sarcoma virus they find three DNA polymerases, the two that are in untransformed cells and the reverse transcriptase that is in murine sarcoma virus particles. Only this latter activity in the transformed cells is inhibited by antisera against the reverse transcriptase of mouse leukaemia virus.

The moral of this story is plain enough. The DNA polymerase activities detected in normal, transformed and cancer cells with synthetic DNA/RNA hybrid templates are normal cellular enzymes unrelated to the reverse transcriptases of the animal RNA tumour viruses. Furthermore, synthetic nucleic acid templates, useful though they are for characterizing reverse transcriptase once its existence has been proved, are no substitute for the natural, single stranded RNA templates when it comes to searching for the enzyme in new situations.

PENICILLINS

Thirty Years After

from a Correspondent

THE thirtieth anniversary of the introduction of penicillin into medicine was commemorated by a meeting at the Royal Society on May 20 and 21, held jointly with the Royal College of Physicians. After an interesting account by Sir Ernst Chain (Imperial College, London) of the early days in the development of penicillin at Oxford, the first day was devoted to a comprehensive survey of current scientific research relating to β -lactam antibiotics.

Recent advances in biosynthesis were reviewed by Dr N. Neuss (Eli Lilly and Co., Indianapolis) who also described his own use of ^{13}C NMR spectroscopy to determine the pathway by which acetate is incorporated into the cephalosporin C molecule. Professor D. H. R. Barton (Imperial College, London) discussed the mechanisms involved in the chemical conversion of penicillin sulphoxides, by way of the corresponding sulphenic acid, to cephalosporin-type molecules. Elimination of the 5-carbon fragment to give the intact β -lactam ring in excellent yield can be achieved by reaction of the dehydropyran derivative of the sulphenic acid with diazomethane, followed by zinc dust reduction.

Other topics discussed were the mode of action of the penicillins, the development of bacterial resistance and immunological problems. Penicillins kill bacterial cells by irreversibly inactivating a transpeptidase involved in the last reaction in the synthesis of cell walls. The question whether the inactivation is a consequence of the penicilloylation of transpeptidase has been investigated by Dr J. L. Strominger (Harvard University). He reported that bacterial cells contain from two to seven components which bind radioactive penicillin, and he suggested that the rapid uptake component is likely to be responsible for the killing of bacteria; this may be the transpeptidase.

The clinical day of the meeting was concerned with the impact of antibiotics, in particular the penicillins, on the treatment of infectious disease. The dramatic decline in mortality in many instances after the introduction of penicillin was illustrated by the study on osteomyelitis reported by Dr P. C. Fleming (Hospital for Sick Children, Toronto). Although penicillin-resistant strains of staphylococci emerged in the 1950s, these have been successfully treated with the newer semi-synthetic penicillins and cephalosporins.

The changing pattern in the distribution of infecting organisms and their antibiotic sensitivity was stressed by several speakers. Professor J. P.

Sandford (Texas University) discussed the increasingly important clinical problem posed by generalized Gram-negative bacillary infections, particularly bacteraemias. These, he suggested are replacing the pneumococci and streptococci of the pre-antibiotic era as the largest single group of life-threatening infections. Dr L. Weinstein (New England Medical Center, Boston) spoke of the altered distribution of organisms in bacterial endocarditis. There has been an increase in the number of cases caused by enterococci and a sharp rise in endocarditis caused by unusual organisms such as *Salmonella* and fungi.

Finally, a note of caution was sounded by Professor R. E. O. Williams (St Mary's Hospital Medical School, London), who pointed out that the body's own defence mechanisms can be damaged by antibiotic therapy. Elimination of bacterial flora from the gut, for example, could result in inter-species substitution or the risk of super-infection.

GLASS SWITCHES

Ovshinsky Hot and Cold

from our Materials Science Correspondent

FEW recent developments in technology have given rise to such vigorous—occasionally passionate—scientific argument as the electric switches based on chalcogenide glasses (Ovonic devices), originally discovered by Ovshinsky in America. There are two types of switches: the “threshold switches”, which can only remain in the switched-on state while current flows, and the “memory switches” which, like computer memories, remain on or off in the passive state until kicked into the converse condition. The unique feature of the whole episode is that Ovshinsky's devices were purely empirical; he is not a trained scientist and at first did not pretend to understand how they worked. Solid-state electronics is the archetypal science-based field of technology, and neither physicists nor electronic engineers have

Gamma Rays under Scrutiny

COSMIC gamma rays are the subject of two articles in next Monday's *Nature Physical Science*. But the energies involved are very different in the two cases—one deals with background flux at energies of a few MeV, the other with possible pulsed emission from pulsars at energies in excess of 10^{13} eV.

F. W. Stecker *et al.* are concerned principally with the gamma ray flux at energies between 1 and 6 MeV, and they take issue with previous proposals that these gamma rays are produced within the galaxy itself. Some of the data on which they have based their arguments were obtained by Vette *et al.* who used a scintillation counter in a small satellite to measure the spectrum of the total flux between these two energies. The crux of the matter is that Vette *et al.* were unable to measure the gamma ray angular distribution, and so could come to no definite conclusions about the origin of the gamma rays. Stecker *et al.* constructed an integral energy spectrum for the galactic disk, and show that it is unreasonable to assume that 1 to 6 MeV gamma rays are galactic.

They also made use of an integral measurement of the flux from the galactic disk above 100 MeV made by Clark *et al.*, and an upper limit of the integral flux at 50 MeV proposed by Fichtel and Kniffen. A theoretical spectrum consistent with both the flux at 6 MeV and the upper limit at 50 MeV turns out to be quite steep, but inconsistent with the Compton scattering mechanism which is thought likely to generate galactic gamma rays with

energies between about 10 and 200 MeV. In particular, the Compton scattering spectrum is too steep to explain the data at 50 and 100 MeV, and not steep enough or of sufficient intensity to account for the spectrum between 6 and 50 MeV, which is required if the 1 to 6 MeV gamma rays are of galactic origin. All this suggests that the 1 to 6 MeV gamma rays are extragalactic and highly isotropic; Stecker *et al.* emphasize that this will constitute a very large background on which point sources emitting gamma rays in this energy range will be superimposed. Detection of weak sources will be very difficult with the rather crude directional instruments available at present.

The same issue of *Nature Physical Science* also contains an attempt to detect pulses of gamma rays from pulsars by Chatterjee *et al.* Their technique is to look for the Čerenkov light emitted by the electron cascades generated in the atmosphere by very energetic gamma rays ($>10^{13}$ eV) and to correlate the signals with the pulsed radio emission of the pulsar. The Čerenkov light is detected by large searchlight mirrors and focused onto the photocathodes of sensitive photomultipliers. Chatterjee *et al.* previously described the detection of significant pulsed emission from CP 0950 (*Nature*, **225**, 839; 1970), but they have now repeated their observations on CP 0950 and five other pulsars and find no such effect. They conclude that their original observation must have been a chance occurrence.

taken kindly to a revolution which owed nothing to scientific insights.

The problem is essentially one of understanding how a homogeneous, amorphous blob of material can be reversibly modified by the passage of a current, when no p-n junctions, Brillouin zones or minority carriers crowd the scene. Because the glasses in question have positive temperature coefficients of conductivity, the idea of some kind of thermal runaway triggered by Joule heat has been very popular, but has never succeeded in explaining the entire behaviour of the switches. More recently, however, a related hypothesis has gained favour—the idea of hot but stable conducting filaments, reversible when small currents flow, but irreversible if currents are large enough because then the Joule heat in the filament devitrifies (that is, crystallizes) the glass. When that has happened, only a current pulse large enough to remelt the crystallites and thus turn them back into glass (when the remelted filament is quenched) can wipe out the imprinted memory. This basic model owes much to two scientists at the Central Electricity Research Laboratories in Leatherhead, J. Male and A. Warren, and was outlined by them in the *New Scientist* (47, 128; 1970).

The model has now been put on a scrupulously quantitative basis by K. W. Böer, working with support from both Ovshinsky's own firm and the US Office of Naval Research. His review article (*Phys. Status Solidi*, (a) 4, 571; 1971) is mandatory reading for anybody interested in the field. He starts from the known conductivity/temperature relationship of a typical chalcogenide glass, and works out the stable temperature distributions in conducting filaments of various aspect ratios, under different assumptions as to cooling efficiency at the electrodes. The problem is complex because the temperature, current and field distributions all influence each other. Experimental observations of local infrared emissions from hot filaments in Ovonic devices offer a direct check on the calculated quantities. The calculations, firmly tied to realities based on numerous experimental measurements, prove, probably for the first time, that the filament model of Ovonic action forms a self-consistent theory for the mode of action of both threshold and memory switches.

EARTH'S MANTLE

Why Gravity Anomalies?

from our Geomagnetism Correspondent

ONE of the most important of the unsolved problems in global geophysics is undoubtedly the source of the large-

scale gravity anomalies which, thanks to data from artificial satellites, are now quite well defined up to harmonic degree $n=8$. These anomalies do not, for example, correlate uniquely with topography and thus presumably cannot be entirely attributable to variations in lateral density in the lithosphere. More specifically they show no obvious relationship to the present pattern of continents and oceans. To some extent this is only to be expected. If there is good isostatic equilibrium between elements of the surface topography and the compensating density distribution below, gravity anomalies from this source should not exist. In fact, the lithosphere does give rise to low degree gravity harmonics; but they are weak and in any case bear no relationship to the anomalies observed. Moreover, there is some apparent correlation between some of the gravity anomalies and tectonic activity. For example, over areas of current post-glacial uplift the anomalies tend to be weak, over

the circum-Pacific belt they are positive and over regions of Quaternary volcanism they also tend to be positive. On the other hand, some of the largest anomalies are apparently unrelated to surface topography, tectonic activity or even what is known about the sub-surface structure of the Earth. A source in the lithosphere thus seems most unlikely.

At the opposite extreme the obvious contender in the deeper parts of the Earth is the core-mantle interface, though there is some doubt about whether irregularities upon this boundary could be large enough to produce the observed anomalies. Nor, according to Cook (*The Earth's Mantle*, Academic Press, p. 63; 1967), it is likely that the lower mantle contains density variations large enough to produce the higher gravity harmonics. The asthenosphere seems more hopeful especially as lateral variations in its structure and physical properties are known to exist. But the large-scale variations in the as-

Doubt about Stellar Fields

SOME contradictions arising from recent measurements of the magnetic fields of white dwarf stars are highlighted in an article in next Monday's *Nature Physical Science* by Virginia Trimble, writing from the Institute of Theoretical Astronomy at Cambridge. It is important to have some idea of the strength of white dwarf fields, if only to verify that the large fields of around 10^6 Gauss that are to be expected from the contraction of a main sequence star to white dwarf size indeed occur. If so, astronomers will be confident that the fields of 10^{12} Gauss which seem to be necessary to explain the radio emission from the even smaller pulsars are feasible. But white dwarf fields are hard to measure from their almost featureless spectra, and there has been much excitement in astronomical circles recently over the discovery that measurements of circular polarization can be used to detect high magnetic fields in sources which emit a continuum.

Virginia Trimble's latest contribution arises from an article by George Preston (Hale Observatories) in *Astrophysical Journal Letters* last year suggesting that fields greater than 10^6 Gauss would also result in a quadratic Zeeman effect that would shift spectral lines toward short wavelengths (160, L143; 1970). This effect ought to be detectable, if the fields are high enough, in the so-called DA white dwarfs, which show hydrogen lines of the Balmer series and are the most numerous classification of the white dwarfs. But Preston points out that measurements of white dwarf spectra published by Greenstein and

Trimble do not show this effect, and hence that the fields of these stars, at least, must be less than about 5×10^5 Gauss.

Next Monday's article is a reassessment of Preston's work, in which Virginia Trimble points out that the upper limit of 5×10^5 Gauss is more stringent than justified because any discordant Balmer lines (presumably such as might originate from the quadratic Zeeman effect) were omitted from the Greenstein and Trimble work on white dwarf spectra. Their analysis was directed at the measurement of the radial velocities of white dwarfs from the Doppler shift of the Balmer lines, and any out-of-the ordinary lines would have been ignored.

So the original data were re-examined to look again for the expected effect, and this time signs corresponding to a median field for the stars of 5×10^5 Gauss were detected in the systematics of the Balmer lines. The contradiction occurs because circular polarization measurements have also been made on some of the stars in the list with no positive result, implying fields less than about 5×10^4 Gauss, whereas the Balmer lines for these stars suggest the presence of fields a factor of ten, or more, larger. Virginia Trimble points out that there may be ways of overcoming this inconsistency, by considering precisely what aspect of the stellar field is being observed by the two methods, although it seems that instrumental effects can still not be ruled out in her measurements based on the shift of the Balmer lines.

thenosphere attributable to temperature differences are essentially differences between sub-continental and sub-oceanic regions; and so the problem of the non-correlation of gravity anomalies with the distribution of continents and oceans is relevant here also.

So what is left? In what may turn out to be a major contribution to the literature, Bott (*Earth Planet. Sci. Lett.*, **11**, 28; 1971) has developed the argument that the source of the global gravity anomalies lies in that part of the mantle between about 400 and 1,000 km depth known as the mantle transition zone. In the Jeffreys-Bullen model of the Earth a steep increase of P and S wave seismic velocities occurs in this region. This anomalous gradient is too steep to be interpreted as the result of simple compression and is, in fact, widely regarded as the effect of phase transitions. In what must to some extent be an oversimplification, both the upper and lower boundaries of the mantle transition zone are interpreted in terms of the behaviour to be expected for a mantle of pure olivine—the olivine-spinel phase change and the breakdown of spinel into its constituent oxides, respectively. Each of these transitions would involve a downward increase in density of about 7–10 per cent over and above that caused by compression and the exact position of each would be temperature dependent. Variations in temperature would thus produce variations of depth along each boundary and hence gravity anomalies.

Is there any positive evidence to support this? To some extent the argument is based on probability with respect to other possible anomaly sources; but at the same time there is a body of laboratory data on the behaviour of the various minerals involved from which Bott has been able to deduce that the relevant transitions should be capable of producing the observed anomalies. Thus he calculates that in the vicinity of a geothermal gradient of 1°C km^{-1} , a rise in temperature of 4.5°C would make the olivine-spinel transition move downwards by about 1 km. The resulting expansion would produce an uplift of about 100 m in the overlying asthenosphere and lithosphere; but this would disappear in a very short time as a result of the outward flow from the asthenosphere. The long-term result would then be a downward migration of part of the olivine-spinel transition by 1 km without significant effect on the overlying material, thereby producing a negative gravity anomaly of about -10 mgal . And because the viscosity in the transition region is probably at least an order of magnitude higher than that in the asthenosphere, the undulation in the transition boundary, and hence the anomaly, would persist for at least 10

million years and possibly even 100 million years.

In practice the maximum temperature anomaly required anywhere to produce all of the observed gravity anomaly patterns is several tens of degrees. But because at least some of the gravity anomalies will be produced by density variations elsewhere in the Earth the real temperature anomaly at any point along the olivine-spinel transition should be somewhat less. The really interesting point, though, is that these comparatively small temperature differences would hardly have much effect on heat flow or seismic delay times; and so these phenomena would not be expected to, and in fact do not, correlate with the global gravity pattern. At this depth only the gravity field is sensitive to structure.

Many of these considerations also apply to the lower transition boundary (spinel to oxides); but because the temperature-pressure relationship is now negative a temperature increase will produce an upward migration of the boundary and thus a positive gravity anomaly. As a result a temperature fluctuation over the whole transition zone will produce two gravity anomalies of opposite sign and thus have little net effect. In the real situation the upper boundary is likely to be the more significant, however, if only because the large temperature variations in the asthenosphere are sure to have a greater

influence on the closer, upper transition.

As Bott points out, whether the upper transition gives rise to a negative or positive gravity anomaly in any given case depends on whether the temperature there is caused to rise or fall. If mantle convection is limited to the asthenosphere heat will gradually rise throughout this layer producing cooling near the base. Persistent convection above a particular part of the olivine-spinel transition is thus likely to lead to cooling and a positive gravity anomaly.

In a region where mantle convection is not active, however, radioactive heating may produce a negative anomaly. This, Bott suggests, may explain the negative anomalies over Precambrian shields. But in the sense that convection currents must be related in some way to the distribution of continents and oceans, should there not then be a correlation between the latter and gravity anomalies? This dilemma is neatly solved. If anomalies can persist for 10–100 million years some of the anomalies currently observed could well be related not to present but past convection currents. Anomalies produced 10–100 million years ago would therefore not be expected to correlate with the present distribution of continents and oceans but only with the older distribution which obtained before seafloor spreading and continental drift changed it.

Sialic Acid and the Immunogenicity of Cells

MOST animal cells have under physiological conditions a considerable net negative charge which is largely attributable to sialic acid. Currie and Bagshawe put forward the hypothesis (*Brit. J. Cancer*, **22**, 843; 1968) that decreasing the negative charge on the surface of normal and malignant cells by digestion with neuraminidase might increase their immunogenicity, and several investigations have suggested that this is often true of cancer cells. Currie *et al.* have also reported (*Nature*, **219**, 191; 1968) that mouse trophoblast after neuraminidase treatment immunizes allogeneic recipients, but there are indications that normal pregnancy can immunize mothers against foetal allo-antigens (Hellström *et al.*, *Nature*, **224**, 914; 1969), so sialic acid does not entirely block immunization by placental cells.

In next Wednesday's *Nature New Biology*, Simmons *et al.* present evidence that treatment of normal mouse spleen cells with neuraminidase likewise increases their immunogenicity as reflected in the time of rejection of a skin graft later made from the same donor strain. The increased immuno-

genicity was observed when the two strains had a major histocompatibility difference, but was more marked when the histocompatibility difference was not H-2.

Why cells become more immunogenic after neuraminidase treatment is not yet clear. Currie *et al.* suggested that the enzyme treatment "unmasks" antigens on cell surfaces, but this seems not to be true of H-2 sites, as shown by capacity to absorb anti-H2 antibody. But weaker antigenic sites, including the theta antigen which has proved to be a useful marker of thymus-derived lymphocytes, may become more accessible after neuraminidase treatment. Another remarkable property of lymphoid cells treated by the enzyme is that instead of migrating to the spleen and lymph nodes they migrate to the liver and remain there for several hours before passing on to the lymphoid organs (Woodruff and Gesner, *J. Exper. Med.*, **129**, 551; 1969); it is possible that something is acquired during their sojourn in the liver that makes them more immunogenic. Neuraminidase-treated cells are also more deformable and show enhanced phagocytosis.

Plants and Soils as Indicators of Metals in the Air

GORDON T. GOODMAN & T. M. ROBERTS

Department of Botany, University College of Swansea

Simple techniques have been used to monitor the non-ferrous metal content of the air in South-West Wales. Metal concentrations downwind of the Swansea urban-industrial complex are found to be significantly greater than the normal background.

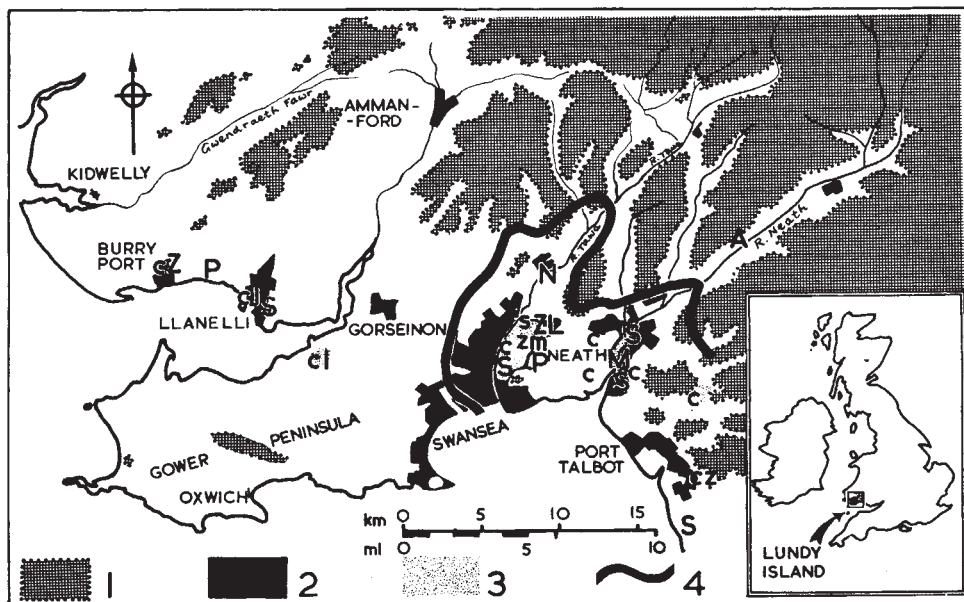
DURING the Lower Swansea Valley Project 1962 to 1969, practical techniques were developed for revegetating bare, industrially derelict land near Swansea which had been affected by the toxic, non-ferrous metals produced by local industry in the nineteenth century¹⁻³. One objective was to eliminate the risk that the exposed, contaminated soils and smelter wastes might periodically dry out and be blown by the wind over a wide area of country occupied by farmland and small industrial towns to the north and east of Swansea, constituting a health hazard to wildlife, crops, livestock and man in the district. The extent to which this was occurring was, however, unknown at the time.

Nineteenth century non-ferrous smelters in the Swansea Valley chiefly produced copper, zinc and lead by processes emitting very large quantities of metal-rich sulphurous smokes and dusts. By contrast, modern processes are much cleaner and at present zinc, lead, cadmium and nickel, but no copper, are being produced. The first four of these metals have been

reported to be chronic health hazards to man and other living organisms even in trace amounts, and soil copper has been used as a baseline for computing soil zinc levels in relation to the incidence of human disease⁴⁻⁹.

The principal object of our study was to obtain some relative estimates of the total aerial burden of metals presumed to originate both indirectly from former industry (that is, through wind-blown contaminated soils and industrial wastes) and directly from present day urban-industrial sources (dusts, smokes and car exhaust). We have not yet made any attempt to separate and assess the contributions of the various emission sources to the total aerial burden. All such sources are known to liberate non-ferrous metals which can affect soils and biota (namely, urban smokes and dusts^{10,11}, wind blown dusts from mining¹², smelting¹³, lead from petroleum in street dusts¹⁴ and air^{15,16}). The geographical spread of aerially borne lead has been demonstrated in the United Kingdom by leaf analysis¹⁷ of privet (*Ligustrum vulgare* L.) and in Sweden for lead and other non-ferrous metals (Cu, Ni, Zn and Cr) in mosses by Rühling and Tyler^{18,19}. Common woodland mosses were already known to accumulate airborne metallic elements very efficiently²⁰ and the survey across Sweden was carried out well away from roads to avoid contamination by lead from petroleum exhaust fumes. Rühling and Tyler demonstrated distinct regional gradients which suggested that the aerially borne metals originated outside Sweden. Analyses of moss material preserved in old herbaria also showed significant increases in metal levels from the mid-nineteenth century to the present, reflecting the influence of human activity. Direct uptake of trace amounts of atmospherically borne metallic elements into

Fig. 1 Map of South Wales to show urban-industrial centres. 1, Land over 180 m OD; 2, chief built-up areas; 3, bare smelter wastes and contaminated soils produced by nineteenth century industry; 4, boundary of *Hypnum* desert around Swansea and Neath. Sites of present industries: A, aluminium working; M, alloy production; N, nickel production; P, large power stations (coal fired during this study); S, iron and steel production; Z, zinc and cadmium production (Swansea) and zinc use (Burryport). Sites of former industries: c, copper smelting; l, lead works; m, general non-ferrous metal production; s, iron and steel production; z, zinc production



leaves has been well established from the environmental behaviour of radionuclides^{21,22}.

A shortage of resources ruled out continuous, direct air-sampling, often in remote places, as a means of obtaining a representative picture of geographical and seasonal variations. Instead, we sampled and analysed for Zn, Pb, Cd, Cu, Ni and Mg in the moss *Hypnum cupressiforme*, in the grass *Festuca rubra* and in surface soils in the Swansea region. Work began in October 1969.

Sampling the Transects

The industrial port of Swansea has a population of about 170,000 and stands at the mouth of the river Tawe in the steep sided Swansea Valley which, like the similarly shaped but even less open Neath Valley, runs in a south-west-northerly direction through the hinterland plateau (about 300 m OD, Fig. 1). Prevailing winds in South Wales are westerly^{23,24}, bringing relatively uncontaminated air from the Atlantic Ocean across the southern coast of Ireland, up the Bristol Channel and along the coastal fringe of South Pembrokeshire, Carmarthenshire and the Gower Peninsula. This region includes Lundy Island and the Gwendraeth Valley with the small town of Kidwelly at its mouth (Fig. 1) and is all open, coastal country almost free of towns and metal industry. By contrast, the area around Swansea and Neath (the Valley mouths) is intensively urban and industrial but beyond this the valleys are all farmland with forest and rough grazings on higher ground. The valleys themselves probably exert wind-corridor and wind-turbulence effects²⁵.

Samples were collected at sites along a broad belt which starts at Lundy Island in the Bristol Channel and runs east-north-east from Oxwich on the Gower Peninsula, along the southern margin of Gower, to the centre of the lower part of

the Swansea Valley (grid reference SS 675967) and was regarded in our study as the "centre" of the urban-industrial complex of Swansea. We refer to this belt as the Gower transect. From this point in Swansea, the belt bifurcates and runs about 26 km up the Swansea and Neath Valleys to form the Swansea and Neath transects which end at Craig-y-Nos and Pontneathvaughan respectively. The Gwendraeth Valley was also sampled from Kidwelly to Gorslas (Gwendraeth transect, 21 km) and together with Gower this serves as a rural comparison with the urban-industrial valleys of Neath and Swansea.

Duplicate samples were collected at each site from positions at least 300 m from any roadway. Soil samples were taken from depths less than 5 cm and leaf material of *F. rubra*, and shoots of *H. cupressiforme* growing 1.5 to 2.5 m above ground on tree trunks, were collected. These tree trunks, principally of sessile oak (*Quercus petraea*), were chosen for their freedom from water tracks. Because of the complete absence of *H. cupressiforme* for several miles downwind to the north of Neath and Swansea (Fig. 1), it was not possible to obtain moss samples at every site. This moss desert is thought to be primarily the result of sulphurous smoke pollution to which mosses are highly sensitive, but metallic fallout may also be important. Care was taken during sampling and subsequent handling to avoid contamination by non-ferrous metals. Soils were dried at 35° C, sieved through a 2 mm stainless steel screen and analysed for Zn, Pb, Cd, Ni and Mg extractable with 0.5 N acetic acid using flame absorption methods; copper was determined after EDTA extraction. Analyses of metals at 1 cm intervals down the depth profiles of several soils along the Gower and Swansea transects showed that the concentrations of Pb, Zn, Cd, Ni and Cu as extracted by boiling concentrated nitric acid were low and that the metals were evenly distributed through the first 10 cm of the relatively uncontaminated profiles of Gower; in the lower part of the

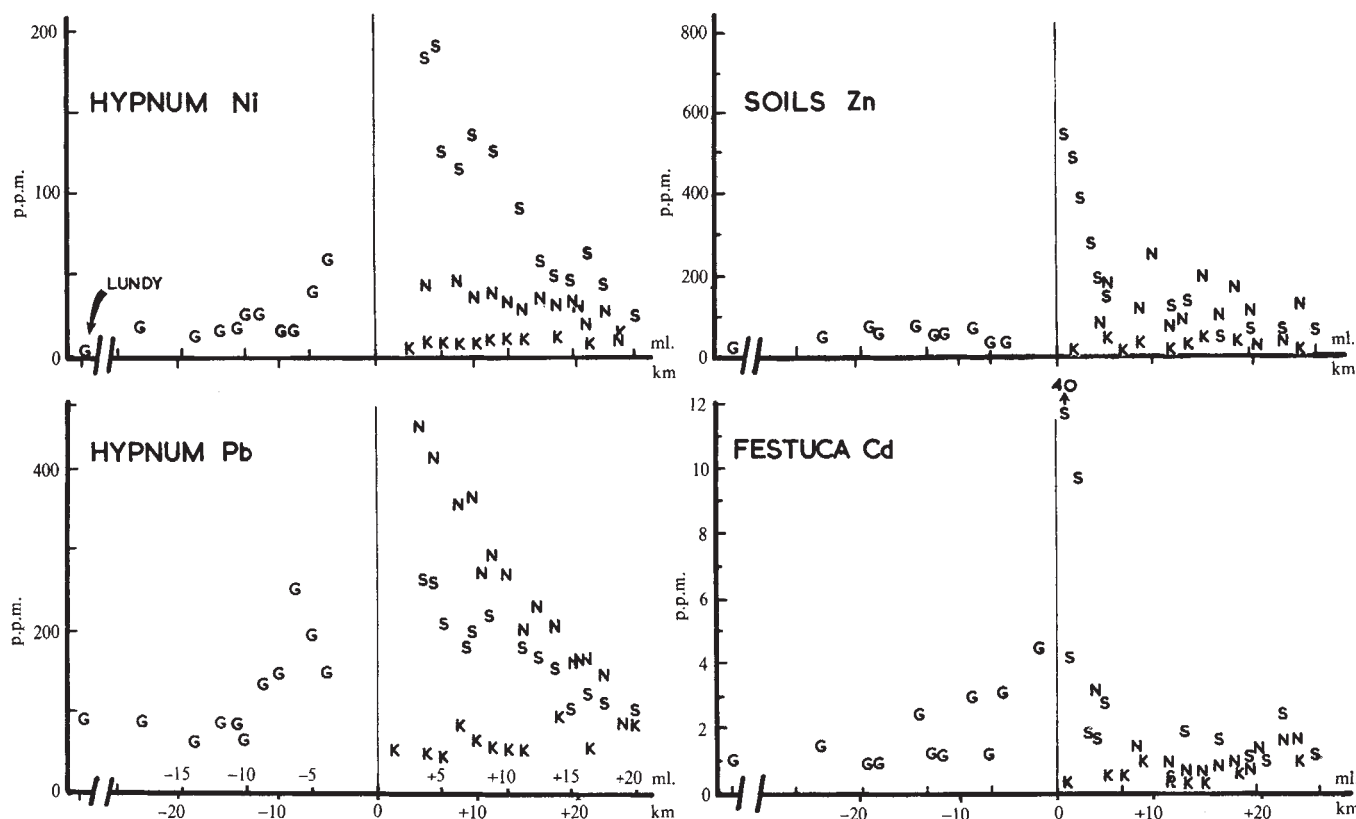


Fig. 2 Metal concentrations in plants and soils along the two contaminated and two uncontaminated transects. N and S, Neath and Swansea transects respectively, distances from the urban-industrial centre of Swansea (Okm, see text) downwind to the respective Valley heads (approximately +25 km); K, Gwendraeth transect, from Valley mouth (Okm) downwind to Valley head (approximately +25 km); G, Gower transect, from Lundy Island (-76 km, left of break in each horizontal scale) downwind to Oxwich (-24 km) and to Swansea centre (Okm). Metal concentration in plants is shown as total p.p.m. dry matter and in soils as 0.5 N acetic acid extractable, p.p.m. air-dry soil.

Table 1 Metal Content of Plants and Soils from Selected Sites along Two Contaminated Transects (Neath and Swansea) and Two Uncontaminated Transects (Gwendraeth and Gower)

Site	Distance (km)			Zn			Pb			Cd			Ni			Cu			Mg		
	H	F	S	H	F	S	H	F	S	H	F	S	H	F	S	H	F	S	H	F	S
Swansea	+1.5	+1.5	+1.5	D	1,190	543	D	814	263	D	40.0	26.0	D	15	20	D	15	160	D	1,260	183
Swansea	+3	+3	+3	D	245	310	D	86	46	D	9.0	8.0	D	15	7	D	15	172	D	1,400	126
Swansea	+8	+6	+6	345	152	150	260	21	14	9.5	2.5	3.0	193	82	234	68	26	214	960	1,170	114
Neath	+8	+5	+5	264	136	105	348	40	17	4.5	3.0	2.0	46	20	2	62	13	210	1,100	1,180	248
Swansea	+25	+16	+16	152	43	45	103	13	8	3.0	1.4	0.3	25	7	6	19	12	80	1,430	1,970	144
Neath	+25	+16	+16	140	60	75	79	16	9	1.0	1.3	0.5	12	8	5	18	12	77	1,640	1,990	187
Gwendraeth	+6.5	+6.5	+6.5	108	25	20	50	5.5	6	1.0	0.7	0.4	6.5	6	3	15	9	35	2,220	2,010	250
Gower	-18	-18	-18	74	25	68	65	12.5	16	1.8	0.8	0.5	10	8	3	11	5	35	1,780	1,650	650
Nävelsjö ³⁰	—	—	—	97	—	—	52	—	—	0.76	—	—	5.5	—	—	9.0	—	—	—	—	—

Gower site is upwind (—) and Swansea and Neath sites downwind (+) of Swansea centre (see text); Gwendraeth site is downwind (+) of Gwendraeth Valley mouth; H, *Hypnum*; F, *Festuca*; S, Soil; D, *Hypnum* desert. Nävelsjö site is in north-east Götaland, southern Sweden³⁰. Metal concentrations in plants are shown as total p.p.m. dry matter and in soils as 10.5 N acetic acid extractable (EDTA extractable for Cu) p.p.m. air-dry soil.

Swansea Valley, however, the concentrations of all these metals were at least about ten times higher at all depths examined and, except for copper, all were strongly concentrated in the top 1 cm.

Moss and grass samples were carefully sorted by hand to remove dead or dying tissue. The living moss shoots and leaf laminae of grass were cut into 1 cm segments with a steel razor, dried for 48 h at 40° C and digested in a 4:1 mixture of specially purified concentrated nitric and perchloric acids. This mixture, or more usually concentrated nitric acid alone, was also effective for animal tissues; the digest was gently evaporated to a small constant volume. Special attention has been paid to the development and standardization of the wet ashing procedure and the subsequent dilution techniques for preparing solutions to spray into the atomic absorption flame. Wet ashing was necessary to avoid metal losses which can be high for lead and cadmium in dry ashing, and the standardization procedure minimized anionic interference which may be very serious in the case of HNO₃, HClO₄ and HCl. Thus all metal standards were made up in solutions which as far as possible simulated the final composition of the diluted digest, and internal standards were used wherever possible.

None of the plant samples were surface washed before digestion. Earlier experiments clearly showed that it was not possible to wash any significant amounts of metal either from moss samples (compare refs. 26 and 27) or grass material when it was obtained outside the moss desert. Grass samples taken inside the desert, however, bore significant quantities of washable metals which increased in the lower Swansea Valley area so that near Llansamlet (grid reference SS688970) as much as 45% of analysed metals could be removed by washing.

As a check on sampling variability, eight adjacent samples of moss and grass were collected from two moderately contaminated sites and analysed as described. Coefficients of variation for the different metals in moss and grass respectively were in the range 7.5 to 8.2 and 5.5 to 9.2. The ten duplicated soil samples along the Gwendraeth transect gave a much wider range, namely 20 to 40.

Metal Content

The results reported here exhibit broadly similar geographical trends for all the metals examined. A selection of the data is shown in Fig. 2 and Table 1.

In soils, zinc values (Fig. 2) are fairly typical of the pattern shown by the other four heavy metals. The Gower and Gwendraeth soils possess similar, uniformly low concentrations of each metal. Within 8 km of Swansea centre, however, soil nickel and cadmium values for the Gower transect gradually rise a little. The concentrations in these two rural transects contrast sharply with the values for the Swansea transect which, except for copper, are about ten times higher at the

starting point and fall off rapidly within the first 8 km downwind, finally approaching Gower levels again after about 16 km. Such differences are thought to be outside the range of natural variation. As existing nickel production is centred 5 km downwind of Swansea centre (Fig. 1), it is interesting to note the delayed increase of soil nickel content to +6 km although the nineteenth century smelter wastes at Swansea centre are known to contain high levels of nickel. A similar but less marked tendency is also evident for copper, but this is inexplicable at present. Soil magnesium values are not clear but seem to be lower in contaminated sites, following the opposite of the pattern for heavy metals.

Table 2 Metal Content of Hay, Grass and Soft Tissues of Horses from Metal Affected and Control Sites

	Zn	Pb	Cd	Cu	Ni
Affected hay 1969 (p.p.m. DM)	166	86	7.6	5.7	5.5
Affected grass 1970 (p.p.m. DM)	360	105	9.9	14.3	13.8
Control grass (Gower) (p.p.m. DM)	58	18	1	6	5
Kidney (p.p.m. wet weight)					
Affected horse	78	40	330	—	—
Control horse	25	3.1	35	—	—
Liver (p.p.m. wet weight)					
Affected horse	143	12	7.5	—	—
Control horse	34	9	1.6	—	—
Lung (p.p.m. wet weight)					
Affected horse	25	1.6	0.3	—	—
Control horse	10	1.3	<0.1	—	—

DM, Dry matter.

The Neath transect displays similar but much less marked trends and greater variability. A delay in the increase of soil copper content is found but not in that of soil nickel which never approaches the levels in the Swansea Valley.

In *Festuca*, the basic content pattern of cadmium (Fig. 2) is similar to that which we have described for soils; the delayed increase in the content of copper and (particularly) nickel is again apparent as for the Swansea soil transect. One unvarying feature of the heavy metal data for the grass is that as the Gower transect approaches Swansea centre (—8 km), metal levels begin to increase steadily; this behaviour is seen only for nickel and cadmium in soils. This may be an indication of current leaf absorption of Zn, Pb and Cu borne aerially by easterly winds^{28,29}.

The very high metal concentrations in *Festuca* leaves just

downwind of Swansea is probably related to the recent recognition of sporadic livestock illness in the area. A farmer living 2 km west of Swansea centre, and inside the moss desert, purchased a horse in October 1969 which was entirely stalled on bales of hay cut from the surrounding fields in the summer of 1969. The horse was put out to grass in the spring of 1970 at which time lead poisoning was clinically confirmed; the horse died in late June. Analyses of hay and horse tissue (Table 2) revealed that the kidney lead content was well above that of the control horse from Gower (killed in a road accident) and within the range recognized as lethal³¹. The renal cadmium level was very high in the affected animal and almost certainly lethal—horses are suspected to accumulate this element irreversibly, as do humans³². The values for lead in 1969 hay and for grass in 1970 were about 100 p.p.m., the accepted lethal value for cows, and horses are recognized as more susceptible¹³.

As expected, the content of magnesium in *Festuca* decreased slowly in the downwind direction along the Gwendraeth transect. Conversely, at the beginning of the Neath and Swansea transects, leaf magnesium content is low but gradually increases downwind to levels equivalent to those at corresponding distances up the Gwendraeth Valley. This inverse effect was suspected for soils and is even more striking in *Hypnum*. Heavy metals may be replacing magnesium in grass foliage.

Because *Hypnum* is an epiphytic moss, it is a more direct indicator of aerial metal burdens than grass, for no uptake from soils can occur and the contribution made by tree bark can be assumed negligible³³. It suffers, however, from the great disadvantage of being sensitive to sulphurous pollution and thus cannot be used to assess aerial concentrations of metals near Swansea and Neath. The data show that Gower and Gwendraeth metal levels are again uniformly low and, except for cadmium and copper, comparable with data from north-east Götaland in southern Sweden³⁰.

As the Gower transect approaches to within 8 km of Swansea centre, metal concentrations begin to increase appreciably, probably because of the easterly winds. The Gwendraeth zinc level, although low, is distinctly higher than on Gower, which suggests a local source.

There are striking differences between the Swansea and Neath transects and the two rural transects in spite of the absence of data in the *Hypnum* desert. Lead levels are high at first but approach Gower levels at about 32 km from the centre. The higher Neath figures cannot be explained, although lead from motor car exhausts may be less easily dispersed in such a trough shaped valley than in the more open Swansea Valley, in which the traffic density is also about half. Nickel values are particularly striking in the Swansea Valley compared with the Neath transect.

The *Hypnum* results are most conveniently summarized as linear regression lines. In Table 3, the constant α gives an indication of the metal level at the upwind end of every transect and the coefficient β measures the change in metal content per

km downwind. The two rural transects show no significant slope for heavy metal sexcept zinc, cadmium and lead in Gower. The concentrations of these elements rose downwind although the Gower sites within 8 km of Swansea centre were omitted from the regression calculations to avoid possible metal contamination from Swansea's urban fringe. The positive magnesium trends in the Neath and Swansea transects contrast with the decreasing values of the other metals which, apart from nickel and lead, show similar general behaviour in both valleys.

Analysis of variance was performed on all *Hypnum* and *Festuca* data; comparison of upwind (Gower transect) and downwind (Swansea transect) data yielded *F* values significant at 0.1% for every heavy metal in moss, and at 1% for each metal in grass, except lead and cadmium (both 5%). This confirmed a marked downwind contamination. Further analysis of variance, performed on the same data divided into ten 4.8 km segments, gave *F* values significant at 0.1% for all metals except copper and lead in *Festuca* (both 1%). The application of Duncan's new multiple range test³⁴ to the *Hypnum* data generally showed that the segment furthest downwind (19 km from Swansea centre) was not significantly different at 1% from the upwind segments, thus indicating a tendency to return to Gower levels. Similarly, the first upwind segment (0 to 4.8 km) in Gower was not significantly different at 1% from the downwind segments, suggesting an upwind contamination effect (probably by easterly winds).

Transplant Experiments

Large logs covered with *Hypnum* were removed from an uncontaminated site near Parkmill, Gower, at the end of June 1970 in an effort to estimate the rate at which *Hypnum* can accumulate metals. They were subsampled for metal analysis and placed 0.8 m above ground in three locations downwind of Swansea (sites 3 to 5, Table 4). The transplanted logs were placed well away from possible sources of contamination and either close to naturally occurring (endemic) *Hypnum* (as in site 3) or, when sited inside the *Hypnum* desert, in habitats judged suitable for the growth of *Hypnum* (sites 4 and 5). Two sets of duplicate samples were collected after 8 weeks; one was analysed unwashed and the other after mechanical shaking in 0.5% surfactant solution. There was no appreciable difference between the metal content of washed and corresponding unwashed samples and so they were averaged, giving four replicates per site. The low metal levels of *Hypnum* from the uncontaminated Gower site 1 had been increased in sites 3–5. Metal levels at site 3 were approaching those in the endemic moss (site 2) although lead seemed to accumulate slower than other metals. The striking accumulation at site 5 of zinc, lead and cadmium to levels at least ten times the greatest natural concentrations ever recorded is particularly interesting. The transplant died after 6 weeks but it may have continued to accumulate metals after death by a passive

Table 3 Regression Constants (α) and Coefficients (β) for Metal Content of *Hypnum cupressiforme* along Transects

	Gwendraeth		Gower		Swansea		Neath	
	α	β	α	β	α	β	α	β
Zn	128	+0.82	56	+4.3 *	349	-12.2 §	359	-11.3 §
Pb	47	+1.4	52	+7.1 ‡	265	-9.7 §	445	-16.5 §
Cd	1.6	+0.02	1.2	+0.06 *	10.3	-0.5 §	7.4	-0.27 §
Ni	6.4	+0.21	11.4	+0.5	188	-9.5 §	47	-1.2 ‡
Cu	13.3	+0.12	9.4	+0.33	92	-3.9 §	61	-2.1 §
Mg	1,975	-23.4 *	1,815	-9.4 *	822	+26.6 §	751	+41.5 §

α is metal content (p.p.m. dry moss) at first sampling point at the upwind end of each transect. β is the slope (p.p.m. km⁻¹ downwind). The last 8 km of downwind end of Gower transect omitted in calculating regression.

*, †, ‡, § are values of β significant at $P < 0.05$, 0.01, 0.005 and 0.001 respectively.

Table 4 Metal Content of Natural, Transplanted and Suspended *Hypnum cupressiforme*

Site	Site near	Transect	Grid. ref. SS	Type of sample	Length of exposure at site (weeks)	Distances from Swansea centre (km)	Zn	Pb	Cd	Ni	Cu	Mg
									(p.p.m.)			
1	Parkmill	G	537896	E	—	—15	92	65	2.5	16	11	1,730
2	Glyn Clydach	N	740997	E	—	+7	400	350	8.7	107	43	1,195
3	Glyn Clydach	N	740997	T	8	+7	360	150	5.6	69	47	1,312
4	Birchgrove	N/S	715984 (D)	T	8	+4	650	353	15.0	215	64	1,222
5	Llansamlet	S	689971 (D)	T	8	+1	4,358	3,732	138	25	78	1,248
6	Ilston Cwm	G	553895	E	—	—14	109	96	2.0	20	14.4	1,735
7	Ilston Cwm	G	553895	B	2	—14	96	96	2.0	20	14	1,976
8	Trebanos	S	717028 (D)	B	2	+8	260	197	6.8	152	83	1,313
9	Coed Gwilym Park	S	721022 (D)	B	2	+6	233	171	5.1	900	261	580
10	Farm	S	688956 (D)	B	2	+2	323	248	10.0	19.5	25	1,100
11	Llansamlet	S	689971 (D)	B	2	+1	4,404	5,617	337	18	47	1,014

G, Gower transect; N, Neath transect; S, Swansea transect; D, site within *Hypnum* desert; E, *Hypnum* sample endemic to site; T, mossy logs transplanted to site; B, moss suspended in mesh bags at site. Distances are upwind (—) or downwind (+) of Swansea centre.

ion-exchange process²⁶. Other features of interest are the relatively low accumulation of nickel at site 5 in comparison with sites 2 to 4—confirming the earlier data and helping to throw light on the emission source for nickel and the decreasing magnesium values in the transplants, which agree with our earlier remarks.

A similar transplant experiment was carried out in December 1970 (Table 4, sites 6 to 11). Clean and dry samples (1.5 g) of *Hypnum* from Ilston Cwm, Gower, were enclosed in nylon mesh bags, pairs of which were suspended by nylon thread 2 m above ground at sites in the Swansea Valley. The farm site (10) was close to the place where the horse mentioned earlier had died. There was little difference in the metal content of *Hypnum* suspended for 2 weeks at its site of origin (7). Samples sited downwind of Swansea centre accumulated large amounts of metal in 2 weeks, however, in spite of the drying and suspension treatment. In fact, the levels were much higher after 2 weeks than after 8 weeks in the first transplant experiment (sites 1 to 5). This may be caused by differences in, for example, the height of samples above ground, the season, wind directions during exposure and other factors which make it advisable to compare only simultaneously exposed and similarly presented samples.

At sites 8 to 10, the zinc, lead and cadmium values increased by a factor of 2 to 5, a striking result after an exposure of only 2 weeks; as expected, magnesium concentrations showed pronounced decreases. The copper and nickel data agree closely with all previous observations which suggest a delayed rise in these elements along the Swansea transect. Copper levels doubled near the nineteenth century deposits of smelter waste at Swansea centre (10) but increased by a factor of 18 to 19 at 6 km downwind (9). The nickel results are even more striking, and the factors are 1 and 45 respectively. These results point to a significant copper emission, of which we have no knowledge at present, occurring in the Swansea Valley between 2 and 6 km downwind of Swansea centre. The copper and nickel values after two weeks at the Llansamlet site (11) do not give any cause to alter this conclusion.

Samples of the *Hypnum* suspended at Llansamlet taken after 4 weeks exposure contained 11,611, 7,166 and 653 p.p.m. of lead, zinc and cadmium respectively. This raises the question of the capacity of *Hypnum* to take up such metals; evidence^{26,27} suggests an ion-exchange process and capacities ~30–100 mequiv. per cent are likely, depending upon the pH and the species of exchanging ion.

Applications and Significance

The results show that surface soils, *Festuca* and particularly *Hypnum* can be used as indicators and integrators of aerial

metallic burdens. Although the region affected by metals around Neath and Swansea seems to have a radius greater than 32 km, the exact extent of the contamination beyond this zone is not clear and should be studied further. Our experience suggests that downwind effects are likely to be important near other urban-industrial complexes and we are already in a position to obtain a national contour map giving very rough but relative estimates of the quantities of airborne metals. Our methods are much more rapid, inexpensive and probably more meaningful than spot-sampling of air by filtration, for which prohibitive resources are needed for a few months' operation. Further information is, however, required about the relative bonding energies of the metals on the exchange surface of *Hypnum* and the modifying influence of rainfall pH. It is also important to know the relative contribution to uptake made by dry deposition (sedimentation and diffusion of various particle sizes) and wash-out processes by rainfall. Data such as those collected in this study could then be interpreted with greater certainty.

The exposure of *Hypnum* in nylon mesh bags or in other more appropriate ways seems to be a particularly promising method of locating emission sources and integrating local metal burdens over a period of weeks or months. This convenient approach would be particularly valuable in the study of metal hazards to wildlife, crops, livestock and man only if the metal concentrations in the moss could be quantitatively related to the total, directly measured metal content of air. Once a relationship had been established by a limited study, the moss technique would be much preferable to direct air sampling.

The apparent inverse relationship between heavy metal uptake and magnesium concentration is of some agricultural interest and may be a factor predisposing livestock to hypomagnesaemia. This and the possible existence of more widespread clinical symptoms of lead or cadmium toxicosis in cattle is worthy of further investigation.

The implications for human health are speculative, but no less important. Some of the medical aspects have been mentioned above^{4–9} but the connexion between age-linked human disorders and the accumulation of heavy metals in human soft tissues has also often been stressed³⁵. It is, moreover, commonly accepted that the absorption of various heavy metals through the lung may be three to ten times greater than through the gut, so that aerially borne metals could be particularly significant. There is also a good deal of evidence to link arterial calcium, magnesium and heavy metals with the differing frequencies of cardiovascular disease in hard and soft water areas^{36–40}. It might be worth considering the broadening of such studies to include aerial metal inter-relationships. The medical considerations certainly emphasize

the need for the establishment of comprehensive aerial metal surveys.

Throughout this study we have been impressed by the significance of the interface relationships between the individual components of the ecosystem—air, soils, plants, animals and possibly even man—and by the need to continue investigations on a broad front to keep an ecological oversight of the biogeodynamics of each metal.

We thank the Natural Environment Research Council for a studentship (T. M. R.), Dr G. Tyler for discussions made possible by a Travelling Fellowship awarded to G. T. G. by the Winston S. Churchill Memorial Trust, and the Royal Society and University College of Swansea for equipment. We also thank Miss J. Stammers, Mrs E. Griffiths, Miss J. Parker and Mr R. O. Roberts for help.

- ¹ Gadgil, R. L., and Gadgil, P. D., *Lower Swansea Valley Project Study Report No. 9* (University College of Swansea, 1965).
- ² Street, H. E., and Goodman, G. T., in *The Lower Swansea Valley Project* (edit. by Hilton, K. J.), 71 (Longmans, London, 1967).
- ³ Goodman, G. T., Pitcairn, C. E. R., and Gemmel, R. P., in *The Ecology of Drastically Disturbed Sites* (edit. by Hutnik, R., and Davis, G.) (in the press).
- ⁴ Kehoe, R. A., in *Industrial Hygiene and Toxicology* (edit. by Patty, F.), 941 (Interscience, New York, 1963).
- ⁵ Lane, R. E., et al., *Brit. Med. J.*, **4**, 501 (1968).
- ⁶ Danielson, L., *Ecological Research Committee Bull.*, No. 6 (Swedish Natural Science Research Council, Stockholm, 1969).
- ⁷ Roe, F. J. C., and Lancaster, M. C., *Brit. Med. Bull.*, **20**, 127 (1964).
- ⁸ Nilsson, R., *Ecological Research Bull.*, No. 7 (Swedish Natural Science Research Council, Stockholm, 1969).
- ⁹ Stocks, P., and Davies, R. I., *Brit. J. Cancer*, **18**, 14 (1964).
- ¹⁰ Stocks, P., Commins, B. T., and Aubrey, K. V., *Intern. J. Air and Water Poll.*, **4**, 141 (1961).

- ¹¹ Purves, D., *Plant and Soil*, **26**, 380 (1967).
- ¹² Egan, D. A., and O'Cuill, T., *Vet. Rec.*, **84**, 230 (1969).
- ¹³ Hammond, P. B., and Aronson, A. L., *Ann. NY Acad. Sci.*, **3**, 595 (1965).
- ¹⁴ Högger, D., *Z. Unfallsmed. BerufsKrankh.*, **2**, 150 (1958).
- ¹⁵ Cannon, H. L., and Bowles, J. M., *Science*, **137**, 765 (1962).
- ¹⁶ Motto, H. L., et al., *Environ. Sci. and Tech.*, **4**, 231 (1970).
- ¹⁷ Everett, J. L., Day, C. L., and Reynolds, D., *Food Cosmet. Toxicol.*, **5**, 29 (1967).
- ¹⁸ Rühling, Å., and Tyler, G., *Bot. Notiser*, **121**, 321 (1968).
- ¹⁹ Rühling, Å., and Tyler, G., *Bot. Notiser*, **122**, 248 (1969).
- ²⁰ Tamm, C. O., *Meddn. Statens Skogsforskningsinst.*, **43**, 1 (1953).
- ²¹ Scott-Russell, R., *Radioactivity and Human Diet* (Pergamon Press, Oxford, 1966).
- ²² Chamberlain, A. C., *Atmos. Environ.*, **4**, 57 (1970).
- ²³ Goodman, G. T., and Gillham, M. E., *J. Ecol.*, **42**, 296 (1954).
- ²⁴ Bilham, E. G., *The Climate of the British Isles* (Macmillan, London, 1938).
- ²⁵ Oliver, J., in *The Lower Swansea Valley Project* (edit. by Hilton, K. J.), 292 (Longmans, London, 1967).
- ²⁶ Rühling, Å., and Tyler, G., *Oikos*, **21**, 92 (1970).
- ²⁷ Clymo, R. S., *Ann. Bot.*, N.S. **27**, 309 (1963).
- ²⁸ Dedolph, R., et al., *Environ. Sci. and Tech.*, **4**, 217 (1970).
- ²⁹ Ter Haar, G., *Environ. Sci. and Tech.*, **4**, 226 (1970).
- ³⁰ Rühling, Å., and Tyler, G., *Rapport No. 10, Ekologiska tungmetallundersökningar* (University of Lund, 1970).
- ³¹ Egan, D. A., and O'Cuill, T., *Vet. Rec.*, **85**, 736 (1970).
- ³² Schroeder, H. A., *Circulation*, **35**, 570 (1967).
- ³³ Barkman, J. J., *Phytosociology and Ecology of Cryptogamic Epiphytes* (van Gorcum, Assen, Netherlands, 1958).
- ³⁴ Duncan, D. B., *Biometrics*, **11**, 1 (1955).
- ³⁵ Schroeder, H. A., *J. Chron. Dis.*, **18**, 217 (1965).
- ³⁶ Crawford, T., and Crawford, M. D., *Lancet*, **i**, 229 (1967).
- ³⁷ Morris, J. N., Crawford, M. D., and Heady, J. A., *Lancet*, **i**, 860 (1961).
- ³⁸ Morris, J. N., Crawford, M. D., and Heady, J. A., *Lancet*, **ii**, 506 (1962).
- ³⁹ Mulcahy, R., *Lancet*, **i**, 975 (1968).
- ⁴⁰ Schroeder, H. A., *J. Amer. Med. Assoc.*, **172**, 1902 (1960).

Developments in Molecular Energy Transfer

A. B. CALLEAR

Department of Physical Chemistry, University of Cambridge, Lensfield Road, Cambridge

Molecular energy transfer is an expanding subject and in this article the chairman of a conference on the topic, to be held at the University of Cambridge between July 19 and July 23, explains where the impetus has come from and what could happen in the future.

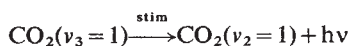
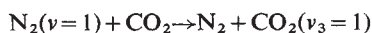
THE subject of molecular energy transfer is undergoing a rapid expansion which is partly a consequence of recent developments and applications of new laser systems—many of which themselves operate by energy transfer mechanisms. Also techniques are now available for the study of certain types of energy transfer between molecules, which until quite recently have been almost unexplored. The first important meeting on the subject was a Gordon Conference held in the United States in June 1969. This meeting was generally considered to be

very successful, and one reason for this was that it brought together scientists of widely different experimental backgrounds, but with a common interest at the molecular level. It is very valuable to have an up to date account of the objectives and views of the various groups. To increase international cooperation in the subject, the decision was taken at the Gordon Conference to hold the second meeting in Europe. This has now been arranged to take place in the University Physical Chemistry Laboratory, Cambridge, and is jointly supported by the Royal Society and the US National Academy of Sciences. The meeting will be run along the lines of a Gordon Conference, with only two principal speakers scheduled for each morning and evening session.

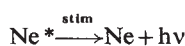
Motions and Transitions

The thermal energy of molecules in gases is made up of translational, rotational, vibrational and electronic energy. All these motions are quantized and transitions between the quantum states can occur by absorption or emission of radiation—the subject matter of spectroscopy. Transitions can also

occur when two molecules collide, and this is the realm of molecular energy transfer. Taking into account the four types of energy, it is evident that there will be at least ten different types of molecular energy transfer. When two molecules collide, for example, transfer of a vibrational quantum may occur from one molecule to the other (a V-V transfer). In this kind of process, the energy remains of the same kind. It is V-V transfer, for example, which produces population inversion in the powerful CO₂ laser.



Electronic energy transfer can be exemplified neatly by the helium-neon laser, in which electronically excited atoms of helium transfer their energy to atomic neon in collision, and thereby set up an inverted population on the manifold of states of the neon atom.



Most energy transfer studies are of a purely fundamental character but a possible outcome—to the great benefit of mankind—could be the application of laser techniques to large scale, thermonuclear fusion.

Intermolecular Forces

One of the focal points at the Cambridge conference will be the nature of the intermolecular forces which control the transfer of energy in collisions. It is known that the rate of vibration to translational energy transfer is generally determined by the short-range, repulsive forces which arise from electron-electron repulsion. Professor J. P. Toennies will describe an experimental investigation of the vibrational excitation of the H₂ molecule by collision with the Li⁺ atomic ion. The basic principle of the experiment is to produce a beam of Li⁺ ions for a very short time, and to direct the beam at target H₂ molecules. From the flight time of the Li⁺ ions inelastically scattered onto an ion detector, the final velocity of the ions can be deduced and hence the extent of internal excitation of H₂ in the encounter. The Li⁺-H₂ system was chosen for study because *a priori* calculation of the potential energy of the collision pair is possible with reasonable accuracy. Results will be compared with a one dimensional, quantum mechanical model for which an exact solution can be computed. Although a considerable gap exists between the unidimensional theory and the real, three dimensional system, it will be extremely interesting to learn to what extent the pattern of final quantum states of the H₂ molecule—and the dependence on initial energy—can be rationalized. The molecular beam experiment is specially important because it can give information on the angular dependence of the energy transfer process.

In other types of energy transfer, such as V-V and R-R (rotation-rotation), where there is only a small change in the total energy of the collision pair, it is becoming increasingly evident that longer range interactions, for example dipole-dipole, play a dominant role. In recent theoretical work on the long-range interactions, Dr R. D. Sharma has interpreted existing results on vibrational relaxation, and has also predicted the behaviour of new experimental systems. He has shown that certain types of vibrational relaxation, for example deactivation of CO(ν₂=1) by H₂, involve a transfer from vibration in the donor to rotational energy in the acceptor. At the Cambridge conference several speakers are expected to cite experimental evidence from ultrasonic measurements and by flash spectroscopy, supporting the dominant role of long range interaction in processes close to resonance.

Interstellar Gas

One of the sessions will be devoted to microwave double resonance and rotational relaxation. During the last five years, the technique of microwave double resonance has been developed and applied to the study of collisionally induced rotational transitions in polyatomic gases. Particularly outstanding has been the investigation by Dr T. Oka of the processes which occur in gaseous NH₃. Because of inversion, the rotational levels of NH₃ are split and the energy of the splitting corresponds to the microwave region of the electromagnetic spectrum. In the double resonance experiment, a particular line is saturated with a powerful signal to equalize the populations of the two components of a rotational state. This disturbance then affects the populations of other rotational levels which are coupled to the pumped states by collisional processes, and these effects can be investigated with a weak probing signal. The existence of various "selection rules" for collisionally induced rotational transitions has been demonstrated experimentally, to reveal a delightful fine structure and order. In NH₃-NH₃ collisions, the dominant interaction is of the long-range, dipole type, which dictates obedience to the electric dipole selection rules. In NH₃-inert gas encounters, the selection rules are quite different. Oka is particularly interested in the rotational relaxation of chemical species which exist in interstellar gas, and will speak on this topic. The consideration of the role of energy transfer processes in astrophysical systems is a new development. So far the microwave double resonance experiment has provided a glimpse of what will ultimately become a large and important subject. One of the speakers at the conference will describe a technique of modulating the pumping signal, to determine not only the identity of particular processes but also their absolute rates.

Measurements of rates of molecular energy transfer have made possible the analysis of laser systems, and there has also been considerable feedback in the application of laser techniques to the study of energy transfer processes. Because of the possibility of Q switching and mode locking, light pulses as short as ~10⁻¹² s can be produced with lasers. With such light pulses, the rates of ultrafast, molecular processes can be studied. The research of Professor G. Porter and his collaborators will be the subject of one of the principal talks at the conference. They have employed a frequency doubled, Q switched, ruby laser pulse to excite some organic molecules to their first singlet states. The singlets undergo an "inter-system crossing" to the lowest triplet states, and with the ultrafast laser techniques it has been possible to examine the newly born triplets which are excited vibrationally, and to measure the rate of energy transfer in collision and the quantity of the excess energy removed per collision. Papers will also be presented on the vibrational excitation of simple molecules with pulsed lasers, and measurements of the relaxation rates by observation with infrared emission and absorption techniques. When the gas is subjected to the laser pulse, excited vibrational levels are overpopulated with respect to thermal equilibrium.

Sessions will also be devoted to measurements with the shock tube, and with ultraviolet fluorescence. In the fluorescence technique the spontaneous emission rate of an electronically excited state provides a built-in clock and the rates of energy transfer processes occurring within 10⁻⁹ s can be measured.

Ten years ago, the only type of molecular energy transfer that was even superficially understood was vibration-translation relaxation. Especially the latter half of the past decade, substantial developments have been achieved in many aspects of the subject. Three chief areas of advance are discernible. a comparison of experiment with exact theory, the recognition of different types of "driving force", and the discovery of selection rules and order in the rotational relaxation of complex molecules.

Territoriality in the White Rhinoceros (*Ceratotherium simum*) Burchell

NORMAN OWEN-SMITH

Natal Parks Board, PO Box 662, Pietermaritzburg, South Africa

The white rhinoceros maintains a territorial system based on a space-correlated dominance relationship in which breeding bulls defend individual areas against rivals, but may share them with subordinates.

THERE may be pronounced differences in social organization between closely related species, or even between different populations of the same species. For primates this variability has been correlated with environmental influences^{1,2}, but field data have been too scanty to demonstrate such a relationship for other mammalian taxa. There has, however, been much recent work on the ungulates, among which territoriality has been revealed as a conspicuous behavioural feature³. The rhinoceroses, being one of the most primitive ungulate families, are of special interest.

The Indian rhinoceros (*Rhinoceros unicornis*) in Kaziranga maintains separate grazing and sleeping territories defended by individuals of both sexes, while wallows and main pathways are communally shared⁴. Contradictory to previously held opinions^{5,6} Schenkel^{7,8} reported that the black rhinoceros (*Diceros bicornis*) in Tsavo, Kenya, did not exhibit territoriality. My observations indicate that the white or square-lipped rhinoceros (*Ceratotherium simum*) has a social system based on a very clearly delineated mosaic of adult male territories.

I have investigated intensively the ecology and ethology of the white rhinoceros in the Umfolozi-Corridor-Hluhluwe game reserve complex in Zululand, South Africa. Observations began in 1966, and since November 1968 have continued without interruption to January 1971. The white rhinoceros is an unusually favourable subject for a detailed study of social relationships. Because of their poor vision individuals or groups can be observed for long periods without disturbance. From variations in horn shape and other features, I have been able to recognize all the adults and several immature individuals in the main study population which includes nearly 200 animals.

The general ecology and conservation of the southern white rhinoceros (*C. s. simum*) have been described by Player and Feely⁹, and its current status has been reviewed by Vincent¹⁰. Backhaus¹¹ has reported some features of behaviour in the northern subspecies *C. s. cottoni*.

Territorial Behaviour

Territoriality is exhibited by only a segment (roughly two-thirds) of the adult male population. Their territorial behaviour may be characterized by the four following features.

(i) Range exclusiveness: the ranges of the territorial bulls, as plotted on the basis of numerous sight records, are mutually exclusive and non-overlapping, so that all suitable habitat is divided into a mosaic of territories, each typically about 2 km² in area. A territorial bull rarely leaves the confines of his territory, except when forced to make excursions to water during the dry season.

(ii) Ritualized encounters; two territorial bulls meeting at a common boundary, engage in a tense but silent confrontation. The bulls repeatedly advance towards each other with raised heads, touch horns (Fig. 1), then back apart to wipe the anterior horn over the ground. Occasionally, with lowered heads, they may clash horns momentarily, but otherwise attacking gestures are inhibited. On the rarely observed occasions on which one bull had intruded a short way into the territory of another, the trespasser backed steadily in the face of the territory owner's advance, until their common border was reached, whereupon the two bulls separated.

(iii) Confinement of oestrous cows: a territorial bull encountering a cow which is nearing oestrus, enters into a consort relationship with her which may last for 2-3 weeks. If this cow wanders towards a boundary region, the bull moves ahead, squealing softly, to stand in front of her, determinedly blocking her further advance. If the cow runs off in fright (for example, when disturbed by my presence), the bull does not pursue her more than 100-200 m beyond his boundary. As a consequence of the territorial system, courtship and mating proceed without interference from other bulls.

(iv) Scent marking: territorial bulls exhibit specialized techniques of defaecation and urination. Backwardly directed kicking movements are made before and after defaecation, so that the dung is broken up and scattered. Although defaecation is almost invariably carried out by territorial bulls at one of the numerous (twenty to thirty) dungheaps present on the territory, these dungheaps are also used by other rhinos, and they are distributed throughout the territory. Urination is effected in the form of a fine spray in three to five spasmodic bursts. It is usually preceded by a scraping action of the legs, and frequently also by a wiping motion of the anterior horn over a low bush or the ground. The fine droplets of urine coat the scrapemarks on the ground and the leaves of the bush if one is present. Urination is not related to any special "marking posts", but is carried out repeatedly whenever the bull moves

about and this probably saturates the territory with his characteristic odour. The existence of preputial scent glands has been reported¹², and there is evidence that rhinoceroses are able to recognize the location of territory boundaries by smell.

Related Elements

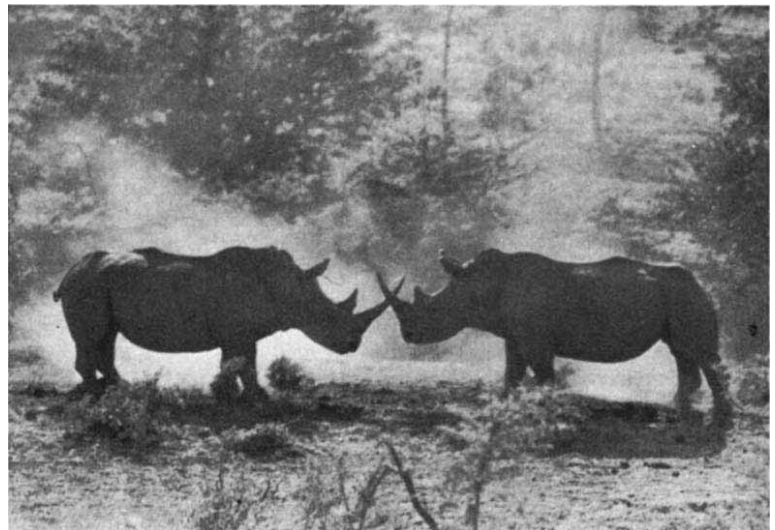
A territorial bull journeying to water attempts to avoid encounters with other rhinos, but, if accosted by a resident territorial bull, he adopts a subordinate defensive stance and bellows and shrieks. When off his territory he does not spray-urinate, but may still exhibit dung-kicking. His whole demeanour changes from confident assertiveness to nervous apprehension.

A bull occupies its territory apparently for several years, so that changes in territory ownership are rare events. From three such observed transitions, however, it is evident that the deposed territorial bull need not vacate his territory, but can remain on it as a subsidiary bull. Following defeat, he immediately ceases spray-urination, and more gradually abandons dung-scattering, initially decreasing the intensity and number of kicks.

Significance of Territoriality

The territoriality of the white rhinoceros is fundamentally a space-correlated dominance relationship between rivals. A territorial bull establishes himself as supremely dominant within the confines of his territory. Direct encounters are not necessary to prevent intrusions. The efficient scent marking system provides a bull with adequate evidence of the presence of others claiming dominance beyond his territorial limits and, in the absence of any motivation to challenge this dominance, a bull prefers to avoid the risk of a meeting. A subsidiary bull is analogous to what is frequently termed a bachelor male in other ungulates. Because all available habitat is divided up into territories, a subsidiary bull is forced to inhabit one of them, and it appears that the owner of the territory eventually becomes habituated to the other's presence. This arrangement may be a result of the relatively high white rhino population densities (about 5/km²) which have built up over much of Umfolozi Game Reserve and now threaten destruction of the habitat. The co-existence of subsidiary and territorial bulls probably represents the initial elements of a rank hierarchy within the territorial structure.

Fig. 1 The horn-touching posture adopted by two territorial bulls during a border confrontation.



Also present in the population are other solitary adult males which do not kick their dung or spray their urine, and which do not associate with cows for any length of time. Although lacking these characteristic features of territorial behaviour, each of these subsidiary bulls essentially confines his activities to the territory of a single territorial bull. A dominance-subordinance relationship exists between a subsidiary bull and the territory owner. If approached, the subsidiary bull exhibits nervousness, but stands his ground with defensive threats; the head is thrust forwards, the ears are flattened, and loud bellows and shrieks are uttered. A brief confrontation may ensue. Often, however, the territorial bull pays no attention to the presence of the other bull, and the two may graze in close proximity in apparent amity. A subsidiary bull sometimes remains nearby when a cow is being mated by the territorial bull, but does not interfere. Subsidiary bulls occasionally wander, but are accosted and sometimes attacked if they meet another territorial bull. Cases are known in which subsidiary bulls have moved elsewhere to become territory holders.

Cow-calf units have independent home ranges extending over 12–15 km², and encompassing seven to eight territories. There is extensive range overlap and they exhibit tolerance and sometimes amity towards other rhinos, with the exception of adult males. Sub-adults associate in pairs or occasionally in larger groups and some seem to wander erratically. The bond between a pair of young males can persist until both individuals are virtually adult in appearance. Cows, non-territorial males and immature animals do not scatter their dung or spray their urine.

A dominance relationship is a means of ordering competition for essential resources. One must therefore enquire in this case, which resource? Food is evidently of no great significance, because a territory owner will readily share his food reserves with a subordinate bull, and also, neither cows nor sub-adults show territorial behaviour. What a bull lacking a territory forgoes is the opportunity to reproduce. The territoriality of the white rhinoceros may thus be described as a system for ordering specifically reproductive competition among males. Its primary function within the population seems to be to increase the reproductive efficiency of prime bulls by reducing the incidence of injury-inflicting combat.

These statements can probably be broadened in scope to apply, with the exception of the Indian rhinoceros, to all other ungulates in which territoriality has been identified, and perhaps to any species in which territoriality is restricted to adult males. In evolutionary terms, territoriality of this type would be favoured over other possible systems for ordering reproductive competition, such as intra-group rank hierarchies (for example, Carmargue wild cattle¹³), or a moving dominance associated directly with a female harem (for example, caribou¹⁴). In this context, its favoured features are: (a), settled range occupancy as opposed to nomadism; (b), low aggregative tendencies; (c), a broadly defined mating season; (d), moderate population densities (low population densities lessen the likelihood of direct competition between rivals and therefore the need for territorial exclusion, high densities may result in the superimposition of a rank hierarchical system).

Alone among the territorial ungulates studied so far, the

Indian rhinoceros, with its system of food territories, seems exceptional. As it also has a peculiar mating system¹⁵ this species warrants a more detailed investigation. The reported lack of territoriality in the black rhinoceros^{7,8} is open to question. As this study has indicated, intensive observations are necessary to distinguish non-territorial and territorial males, which, if they inhabit the same range, can obscure the spatial pattern expected of territoriality.

This work has been supported by the Natal Parks Board, the University of Wisconsin Alumni Research Foundation, and the US National Science Foundation. The article has benefited from the critical comments of Professor John T. Emlen.

Received November 19, 1970; revised March 11, 1971.

- ¹ Crook, J. H., and Gartlan, J. S., *Nature*, **210**, 1200 (1966).
- ² Gartlan, J. S., *Folia Primatol.*, **8**, 89 (1968).
- ³ Estes, R. D., *Z. Tierpsychol.*, **26**, 284 (1969).
- ⁴ Ullrich, W., *D. Zool. Garten*, **28**, 225 (1964).
- ⁵ Hediger, H., *Bijdr. t.d. Dierkunde*, **28**, 172 (1949).
- ⁶ Ripley, S. Dillon, *Ecology*, **39**, 172 (1958).
- ⁷ Schenkel, R., *Z. Tierpsychol.*, **23**, 593 (1966).
- ⁸ Schenkel, R., and Schenkel-Hullinger, L. M., *Ecology and Behaviour of the Black Rhinoceros* (Paul Parey, Berlin, 1969).
- ⁹ Player, I. C., and Feely, J. M., *Lammergeyer*, **1**, 3 (1960).
- ¹⁰ Vincent, J., *Lammergeyer*, **10**, 12 (1969).
- ¹¹ Backhaus, D., *D. Zool. Garten*, **29**, 93 (1964).
- ¹² Cave, A. J. E., *Mammalia*, **30**, 153 (1966).
- ¹³ Schloeth, R., *Z. Tierpsychol.*, **18**, 574 (1961).
- ¹⁴ Lent, P. C., *Anim. Behav.*, **13**, 259 (1965).
- ¹⁵ Ripley, S. Dillon, *Ecology*, **33**, 570 (1952).

Trophic Interaction between Cloned Tissue Culture Lines of Nerve and Muscle

A. JOHN HARRIS*, STEPHEN HEINEMANN,
DAVID SCHUBERT & HELGI TARAKIS

The Salk Institute, PO Box 1809, San Diego, California 92112

Tissue culture of neural tumour cells with skeletal muscle provides a model for the early stages of synapse formation. Functional synaptic transmission, however, has not yet been seen.

AN important generalization underlying present understanding of the development and function of the nervous system is the great orderliness and specificity of interneuronal and neurone-endorgan connexions. Little is known of the physiology of formation of these connexions, either in developing embryos or during regeneration of cut nerves in adults. One indication of how a cell signals its willingness or unwillingness to accept innervation comes from work on muscle by Miledi, who described a striking correlation between the distribution of chemoreceptors on a presumptive postsynaptic cell surface, and the ability of that cell to accept a new synapse^{1,2}. Thus damaging a muscle fibre at a discrete point, which causes a local hypersensitivity to acetylcholine³, also locally removes the barrier to hyperinnervation of that muscle fibre, so that a new synapse can be formed at the hypersensitive area². Recent studies of regenerating synapses on parasympathetic nerve cells in the frog heart⁴ provide a similar correlation between chemosensitivity of a neuronal surface and the receptivity of that neurone to innervation, showing that this relationship is not unique to muscle.

Briefly summarized, the phenomena that seem common to synapse formation both on skeletal muscle and parasympathetic nerve cells¹⁻⁴ are as follows: (a) the noninnervated post-

synaptic cell is more or less uniformly sensitive to neural transmitter; (b) contact with an ingrowing nerve is followed by removal of this generalized sensitivity and the development of local sensitivity at the point of contact, probably before there is any functional synaptic transmission¹; (c) other nerve fibres are now unable to make synapses on the insensitive region of that cell; and, finally, the synaptic contact is enlarged and normal function becomes possible.

Our aim has been to develop a simple system in which synapse formation can be studied under controlled conditions *in vitro*. It has long been known that synaptic connexions can be induced to form within primary cultures of tissue explants or disaggregates⁴⁻⁸, but there are still many cell types in such cultures, making it difficult to undertake the detailed biochemical studies necessary to define the molecular events responsible for the formation and observed specificity of synapses. We have chosen to study the interaction between pure (cloned) continuous tissue culture lines of nerve and muscle. The nerve cells are from the C 1300 mouse neuroblastoma, clone C1A⁹, and the muscle cells from a cell line of rat skeletal myoblasts, clone L-6¹⁰, given us by Dr D. Yaffe.

The nerve-like properties of the neuroblastoma have been described in moderate detail^{9,10-13}, but the physiological properties of the muscle cell line have not been described. A detailed description of these cells will be presented elsewhere; briefly, the cell line is carried in the form of myoblasts, which grow in a monolayer on tissue culture dishes. After becoming confluent the cells begin to fuse, forming multinucleate myotubes which later develop into striated muscle fibres. The fibres begin to beat spontaneously late in the myotube stage, before striations appear. The development of electrical excitability is similar in many respects to that recently described in developing tunicate muscle¹⁴. Individual myoblasts have resting potentials of about -70 mV, and behave in an electrically passive manner when depolarizing or hyperpolarizing

* Present address: Department of Physiology, University of Otago, Dunedin, New Zealand.

current pulses are passed into them. If physical contact is seen between myoblasts they are always electrically coupled.

Fusion of the myoblasts to form binucleate or multinucleate cells is accompanied by a fall in resting potential, to about -50 mV. Depolarizing stimuli produce little active response, but action potentials can be elicited by anode-break stimulation (that is, by hyperpolarizing the cell, say to -150 mV, and then abruptly terminating the stimulus). An action potential evoked in this manner has a duration of as much as 100 ms, and is followed by prolonged hyperpolarization. With further time in culture, and a greater degree of fusion, the resting potentials increase, so that action potentials can be evoked with depolarizing stimuli. At about this time they appear spontaneously, with a steady frequency of about 1/s. Some time later the cells are able to contract, and most multinucleate cells in a culture dish beat spontaneously so long as adequate culture conditions (in particular provision of Ca^{2+}) are maintained.

Myoblasts, myotubes, and striated muscle fibres are all sensitive to acetylcholine (ACh). A brief pulse of ACh applied to a myoblast with an iontophoretic pipette gives rise to a long lasting hyperpolarization (15–30 s) with a concomitant increase in membrane conductance. After fusion has begun,

cells with several nuclei may still show the slow hyperpolarizing response, but many have a dual response: a fast depolarization, followed by the slow hyperpolarization. Finally, all cells become uniformly sensitive to ACh so that wherever it is applied a fast rising (< 50 ms time to peak) depolarization results.

The experimental interaction of neuroblastoma and muscle cells is complicated by the fact that both cell lines have been selected for growth rather than the ability to maintain a stable differentiated state in culture, and we have not yet found a set of conditions where both cell types can be maintained stably for long times. For the experiments described here we grew myoblasts to an appropriate density in Falcon tissue culture dishes in Dulbecco modified Eagle's medium¹⁵ supplemented with 20% foetal calf serum, and then lowered the serum concentration to 1% to promote fusion. Once an adequate number of fused cells was seen in a dish, after about a week, neuroblastoma cells taken from suspension culture were plated on top of the muscle cells, using fresh medium supplemented with 1% foetal calf serum and 5 mM CaCl_2 . Within 1 or 2 days the neuroblastoma cells began to extend processes and to contact the muscle cells.

Both myotubes and myoblasts were present in these cultures,

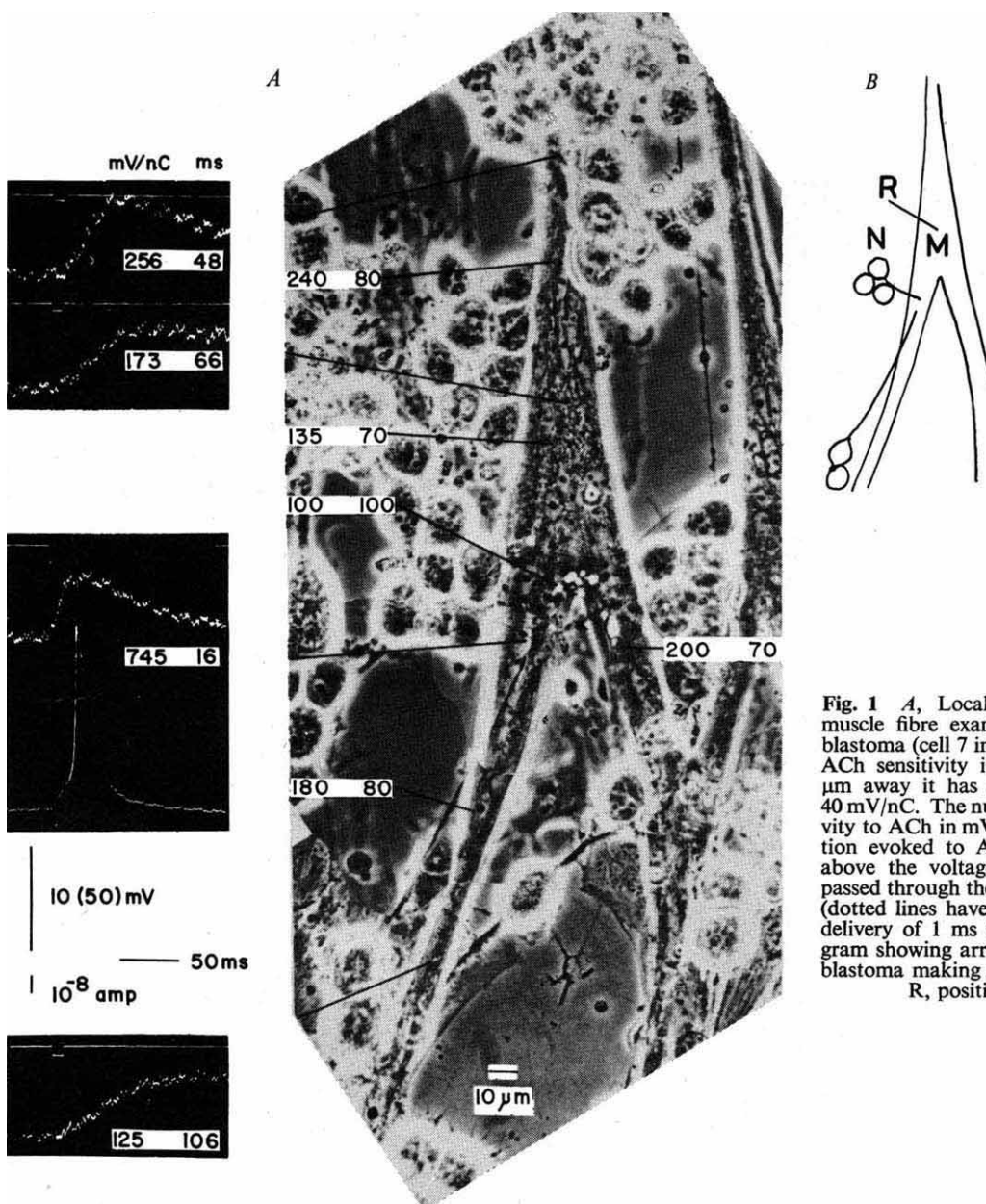


Fig. 1 *A*, Localization of ACh sensitivity on a muscle fibre examined 7 days after adding neuroblastoma (cell 7 in Table 1). In the region of contact, ACh sensitivity is about 200 mV/nC, while by 50 μm away it has fallen to the background level of 40 mV/nC. The numbers by each trace indicate sensitivity to ACh in mV/nC (that is, the ratio of depolarization evoked to ACh current delivered). The lines above the voltage records show the current pulse passed through the ACh pipette to evoke the response (dotted lines have been added to indicate the time of delivery of 1 ms current pulses). *B*, Schematic diagram showing arrangement of cells in *A*. N, Neuroblastoma making contact; M, muscle cell (myotube); R, position of recording electrode.

but there were also a few striated muscle fibres. In control cultures without neuroblastoma the larger myotubes were at the stage at which they gave depolarizing responses to ACh, and a few were giving spontaneous action potentials, but were not contracting. The interacting cells were examined using microelectrodes for intracellular recording and iontophoretic application of ACh. The culture fluid was covered with a layer of paraffin oil to prevent evaporation and to maintain the CO_2 content of the medium, and the cells were maintained at 32°C in a warmed holder on the stage of an upright microscope. Cells were visualized under a $\times 40$ phase objective, and the microelectrodes positioned at a shallow angle so as to move freely under the lens.

Near points of contact with a neuroblastoma process, however, a 1 ms pulse would often produce a depolarization of sufficient intensity to evoke a muscle action potential. This local chemosensitivity had an intimate relation to the neuroblastoma terminal, and fell off rapidly to either side of it. An example is shown in Fig. 1 where points were tested at brief intervals away from the contact. With the electrode $30\ \mu\text{m}$ away the response was only 60% of that near the neuroblastoma process, and by $50\ \mu\text{m}$ away the sensitivity had fallen to the level found uniformly distributed over the rest of the muscle.

Fig. 2 illustrates another example of localized sensitivity. In this case a 1 ms pulse of ACh applied to the contact point gave rise to an action potential, while less intense pulses

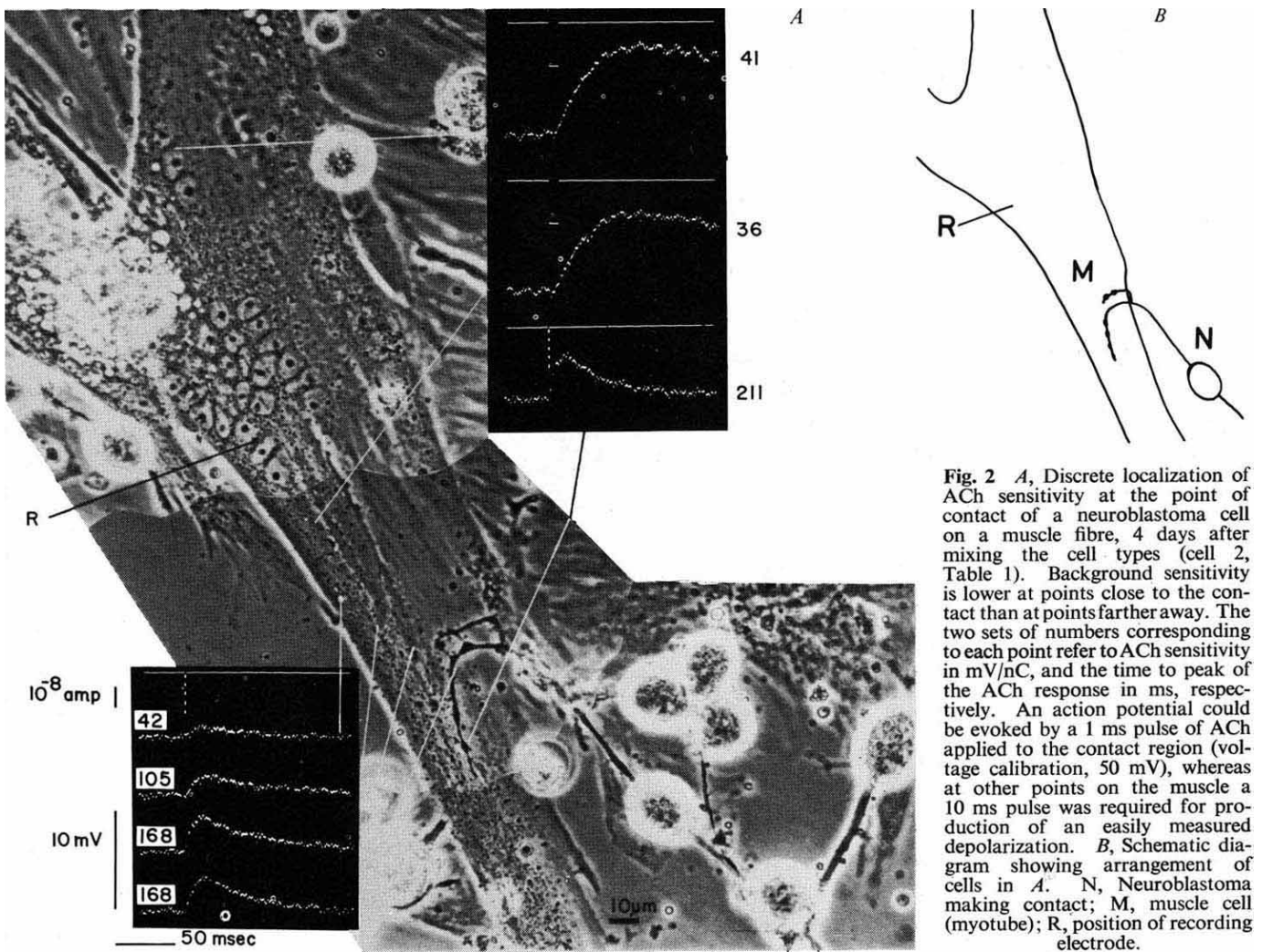


Fig. 2 *A*, Discrete localization of ACh sensitivity at the point of contact of a neuroblastoma cell on a muscle fibre, 4 days after mixing the cell types (cell 2, Table 1). Background sensitivity is lower at points close to the contact than at points farther away. The two sets of numbers corresponding to each point refer to ACh sensitivity in mV/nC , and the time to peak of the ACh response in ms, respectively. An action potential could be evoked by a 1 ms pulse of ACh applied to the contact region (voltage calibration, 50 mV), whereas at other points on the muscle a 10 ms pulse was required for production of an easily measured depolarization. *B*, Schematic diagram showing arrangement of cells in *A*. N, Neuroblastoma making contact; M, muscle cell (myotube); R, position of recording electrode.

The dish was searched for muscle cells which had the process of a neuroblastoma cell terminating on their surface. Such a muscle fibre was then impaled for intracellular recording, and the distribution of chemosensitivity on its surface mapped by applying brief iontophoretic pulses of ACh from a micropipette placed at different points along its length. The ACh-sensitivity of each spot is expressed as the magnitude of the response to each iontophoretic pulse ($\text{mV depolarization/nCoulomb of ACh current}$) and the time to peak of the depolarization. Both these parameters should reflect the local density of ACh receptors¹⁶.

The most striking effect of interaction, seen most frequently 4 days or later after mixing the cells, was a region of increased chemosensitivity restricted to the vicinity of the contact. ACh current pulses of 10^{-8} A and 10 ms duration were usually necessary to obtain a detectable response from a muscle fibre.

produced a fast rising depolarization. ACh sensitivity again fell off rapidly as the ACh pipette was moved away from the contact.

After recording from cells we occasionally tried to pull them apart to test the mechanical strength of the neuroblastoma-muscle contact. Where there had been a local sensitivity at the contact, the cells were found to be firmly attached to one another, so that pulling the neuroblastoma process tore the muscle membrane away. On the other hand, regions of apparent contact were seen where there were no inhomogeneities in muscle chemosensitivity, and, in such cases, probing with the electrodes often indicated a lack of mechanical attachment.

A summary of the results from experiments on eleven cells examined at various times up to 11 days after interaction is shown in Table 1. The difference between the contact sensitivity and the diffuse sensitivity varied over the range $\times 2$ to

$\times 25$, with most cells having about a five-fold difference. The times to peak of the responses from contact regions are similarly from three to five times faster than those from the extra-junctional regions.

Muscle cells examined early, 2–4 days after mixing with neuroblastoma, had a lower sensitivity to ACh than muscles in control cultures. Sometimes, as illustrated in Fig. 3 and Table 2, a region of lowered chemosensitivity was clearly associated with the contact region of a neuroblastoma neurite. Later, when raised chemosensitivity was associated with the contact point, regions of the muscle surface 50 or 100 μm away were frequently less sensitive than regions at a greater distance (for example, cells 2, 6 and 9 in Table 1). This effect is often seen in normal frog muscles (ref. 17 and unpublished results of R. Miledi).

These experiments show clearly that a discrete region of raised chemosensitivity, similar to that seen at a normal nerve–

muscle junction¹⁶ can be induced on a muscle surface at the point of attachment of a neuroblastoma cell process. While the evidence for a specific effect of neuroblastoma in lowering sensitivity away from points of contact may be less compelling, it seems that the sequence of events seen in the early stages of contact between neuroblastoma and muscle is very similar to those seen in the early stages of synapse formation on muscle or parasympathetic nerve cells¹, that is, first, a general lowering of ACh-sensitivity (near) the contact, followed later by the development of a high sensitivity at the point of contact.

We cannot definitely relate these events in the *in vivo* and *in vitro* systems until chemical synaptic transmission is clearly demonstrated between the tissue culture cells. We looked for synaptic transmission, by recording simultaneously from a muscle fibre and from the cell body of the neuroblastoma cell in contact with it, and then setting up an action potential in the neuroblastoma cell, but saw no clear signs of functional

Table 1 ACh Sensitivity Mapped along the Length of Muscle Fibres contacted by a Process of a Neuroblastoma Cell

Cell	Days	Distance (μm)	Sensitivity (mV/nC)	Time to peak (ms)	Cell	Days	Distance (μm)	Sensitivity (mV/nC)	Time to peak (ms)
1	3	20	100	140	6	6	30	172	50
		0	150*	60			20	35	40
		0	143*	100			0	376*	30
		0	148*	75			30	37	125
		20	162	65			100	180	30
		30	62	90			120	160	35
		100	40	75			150	100	40
		120	73	125			180	52	40
		150	69	125	7	7	245	41	80
		300	65	70			120	42	80
		320	31	80			100	36	65
		350	20	60			70	42	20
		400	13	200			50	105	20
2	4	170	256	48			40	168	16
		130	240	80			0	168*	16
		85	173	66			0	211*	16
		70	135	70	8	11	100	100	20
		20	100	100			120	77	30
		0	745*	16			20	88	75
		50	200	70			0	200*	30
		60	180	80			50	224	28
		115	125	106			100	118	70
							120	208	30
							200	75	75
					9	11	200	189	26
							100	37	50
3	5	0	490*	40			0	334*	30
							0	725*	26
4	5	160	10	80	10	11	180	40	75
		100	15	85			100	110	50
		55	17	75			0	220*	30
		0	296*	30			0	200*	35
		30	32	80	11	11	50	214	60
		80	32	65			0	1,325*	18
		100	27	50			50	20	50
		120	30	40			70	103	30
		150	55	35			150	35	50
5	6	100	190	60			180	40	50
		0	530*	25			200	190*	12
		0	414*	25			350	15	100
		20	274	50					
		80	200	60					
		100	64	50					
		120	130	50					

Distances are measured from an arbitrary point in the contact region.

* Sensitivities near a contact.

transmission in cultures of the type described here. In longer term cultures, we saw three examples in which the first action potential in the neuroblastoma gave rise to a small depolarization in the muscle, while later stimuli were ineffective. We have no evidence to confirm that this transient effect was due to chemical transmission. We have looked in 11 day old cultures for low resistance junctions between muscle and neuroblastoma, and noted their presence, but have not examined earlier cultures in this way (to see electrical coupling, the impedance mismatch between neuroblastoma and muscle makes it necessary to stimulate the muscle, while recording from the neuroblastoma, if a detectable response is to be obtained).

Although this combination of cell types does not occur naturally, synapse formation may still be possible. Sympathetic nerves can form functional synapses on skeletal muscle in the frog¹⁸, and, although this was not seen in rather similar experiments using cats¹⁹, the sympathetic nerves did abolish the fibrillation due to denervation of the muscle.

The simple and controlled nature of our experimental system now places us in a favourable position to determine the mechanism of this intercellular communication process. The local effects of interaction depend on contact, and may also involve the discrete transmission of some chemical from one cell to the other. Clearly, the most exciting possibility is that

Table 2 Region of Decreased ACh Sensitivity associated with the Contact with a Neuroblastoma Neurite at an Early Time (4 days) after Interaction

Distance	Sensitivity	Time to peak
160	78	90
140	72	100
105	52 *	70
80	30 *	75
75	40 *	80
70	26 *	150
65	32 *	150
55	40 *	125
50	42 *	125
35	94	65
20	160	35
0	307 *	45
50	132	65
170	98	75

Two neuroblastoma cells contacted this muscle, one being associated with a region of low sensitivity and the other of high sensitivity. This cell is illustrated in Fig. 3. Distances and sensitivities as in Table 1.

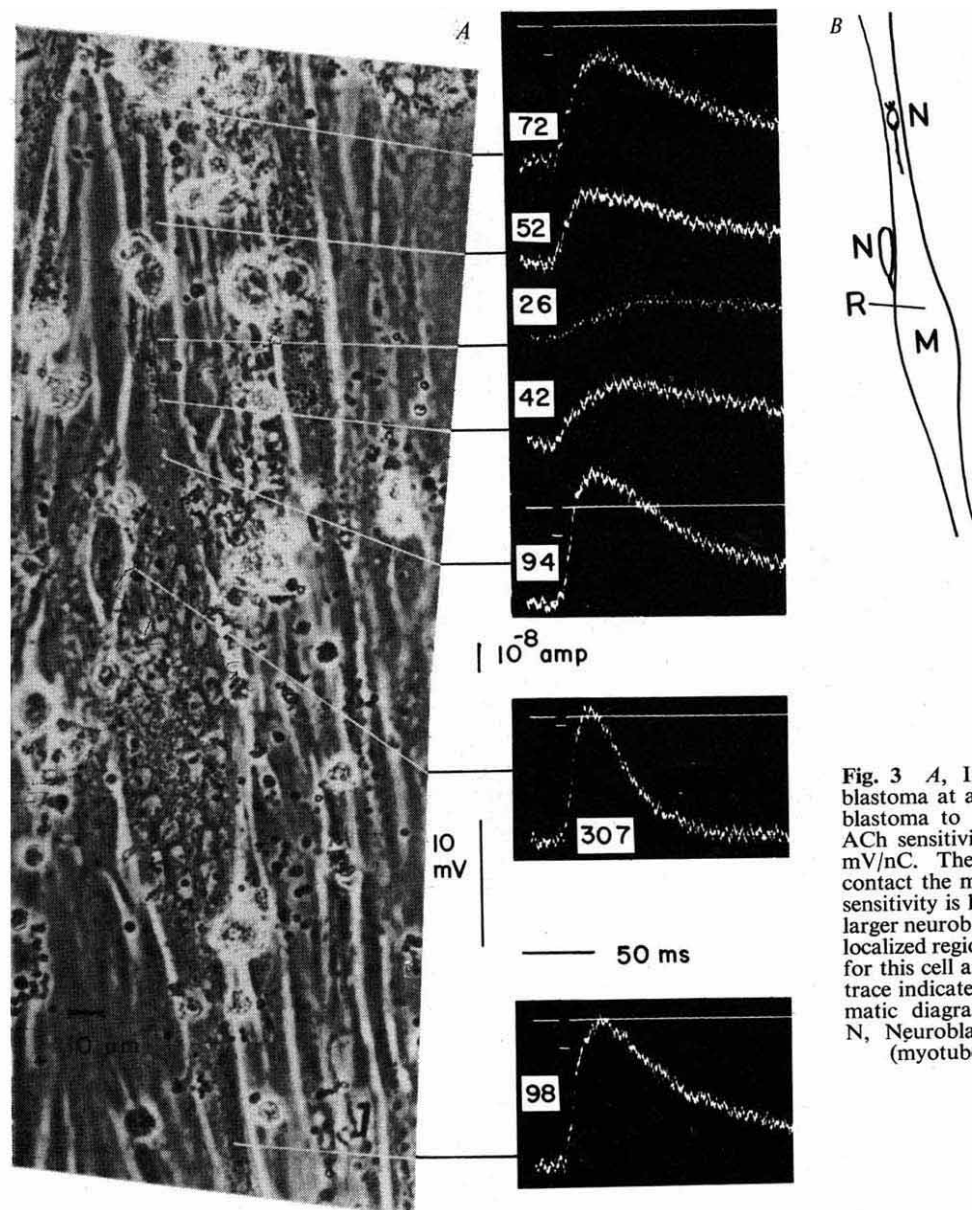


Fig. 3 *A*, Interaction between muscle and neuroblastoma at an early time, 4 days, after adding neuroblastoma to the muscle culture. The "background" ACh sensitivity of the muscle is in the range 70–100 mV/nC. The processes of a small neuroblastoma cell contact the muscle over a 50 μ m length, and here the sensitivity is low, in the range 30–40 mV/nC. Another larger neuroblastoma cell is associated with a discretely localized region of higher sensitivity. The complete data for this cell are given in Table 2. The numbers by each trace indicate sensitivity to ACh in mV/nC. *B*, Schematic diagram showing arrangement of cells in *A*. N, Neuroblastoma making contact; M, muscle cell (myotube); R, position of recording electrode.

this system will provide an easily accessible model with which to study synapse formation, and this study already proves its usefulness as a preparation with which to analyse the mechanisms underlying some of the trophic effects of nerve on muscle²⁰.

This work was supported by grants from the Jane Coffin Childs Memorial Fund for Medical Research (to A. J. H.) and from the Damon Runyon Foundation, the California Cancer Society and the National Institutes of Health (to D. S.). A. J. H. is a postdoctoral fellow of the National Multiple Sclerosis Foundation.

Received March 11, 1971.

- ¹ Miledi, R., *J. Physiol.*, **154**, 190 (1960).
- ² Miledi, R., *Nature*, **199**, 1191 (1963).
- ³ Katz, B., and Miledi, R., *J. Physiol.*, **170**, 389 (1964).
- ⁴ Levi, G., *Erg. Anat. Entw.*, **31**, 123 (1934).
- ⁵ Bunge, R. P., Bunge, M. B., and Peterson, E. R., *J. Cell Biol.*, **24**, 163 (1965).

- 6 Crain, S. M., *Intern. Rev. Neurobiol.*, **9**, 1 (1966).
- 7 Shimada, Y., Fischman, D., and Moscona, Z., *Proc. US Nat. Acad. Sci.*, **62**, 715 (1969).
- 8 Fischbach, G. D., *Science*, **169**, 1331 (1970).
- 9 Schubert, D., Humphreys, S., Baroni, C., and Cohn, M., *Proc. US Nat. Acad. Sci.*, **64**, 316 (1969).
- 10 Richter, C., and Yaffe, D., *Develop. Biol.*, **23**, 1 (1970).
- 11 Augusti-Tocco, G., and Sato, G., *Proc. US Nat. Acad. Sci.*, **64**, 311 (1969).
- 12 Nelson, P., Ruffner, W., and Nirenberg, M., *Proc. US Nat. Acad. Sci.*, **64**, 1004 (1969).
- 13 Harris, A. J., thesis, University of London (1968).
- 14 Takahashi, K., Miyazaki, S.-I., and Kidokuro, Y., *Science*, **171**, 415 (1971).
- 15 Vogt, M., and Dulbecco, R., *Proc. US Nat. Acad. Sci.*, **49**, 171 (1963).
- 16 del Castillo, J., and Katz, B., *J. Physiol.*, **128**, 157 (1955).
- 17 Harris, A. J., thesis, University of London (1968).
- 18 Landmesser, L. T., *J. Physiol.*, **213**, 707 (1971).
- 19 Mendez, J., Aranda, L. C., and Luco, J. V., *J. Neurophysiol.*, **33**, 882 (1970).
- 20 Guth, L., *Physiol. Rev.*, **48**, 645 (1968).

Total Structure of Capreomycin IB, a Tuberculostatic Peptide Antibiotic

B. W. BYCROFT, D. CAMERON, L. R. CROFT,
A. HASSANALI-WALJI, A. W. JOHNSON* & T. WEBB

Department of Chemistry, University of Nottingham

Analytical data suggest that the capreomycins and viomycin are members of the same closely related family of antibiotics.

WE recently proposed structure (I) for the tuberculostatic antibiotic viomycin¹ and, as in previous publications, we noted the similarities between the physical, chemical and pharmacological properties of viomycin and the capreomycin group of antibiotics. The antimicrobial activity of capreomycin is restricted to *Mycobacterium tuberculosis* and, like viomycin, it has found limited clinical application.

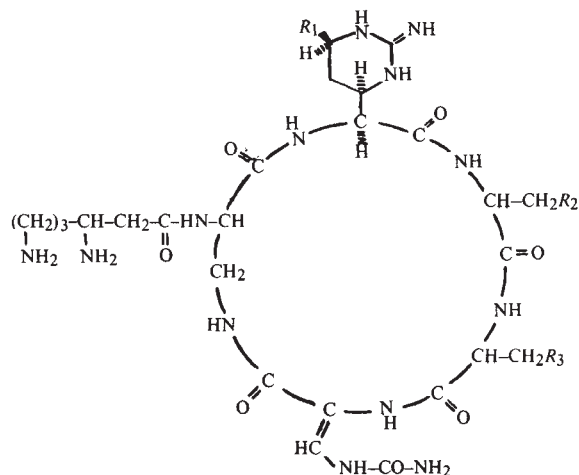
The capreomycins were isolated initially in 1960 from *Streptomyces capreolus*² and were shown subsequently to be a complex of four closely related components, designated capreomycins IA, IB, IIA and IIB³. Separation of the complex on an ion-exchange column and elution with increasing concentrations of aqueous triethylamine sulphate readily yielded capreomycin I and capreomycin II (ratio 9 : 1 respectively), both of which were separated into two components, A and B (ratio approximately 1 : 2 in each case). We have repeated this procedure and have found that although it provides an effective separation of the components, they were all contaminated with triethylamine sulphate. Consequently, the analytical data and molecular weight determinations reported previously³ require revision.

We have concentrated on capreomycin IB as this is the principal component of the complex. Our analytical data and molecular weight determinations on the sulphate, free from triethylamine sulphate, are consistent with the molecular

* Present address: School of Molecular Sciences, University of Sussex, Falmer, Brighton.

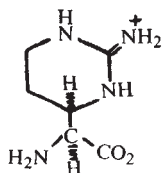
formula $C_{25}H_{44}N_{14}O_{7.2}H_2SO_4$ (molecular weight 850.79) in accordance with our proposed structure (II). Titration of capreomycin IB revealed the presence of four basic groups with pK_a values of 6.2, 8.2, 10.1, and 13.3, but although a strong base, the antibiotic gives a negative Sakaguchi reaction. The Fehling, Molisch, and ferric tests were negative, but positive results were obtained from ninhydrin and biuret reactions.

As originally reported, total acid hydrolysis afforded α -diaminopropionic acid (dapr), alanine (ala), β -lysine (β -lys) and capreomycinidine (cap) (2 : 1 : 1 : 1, respectively)^{2,3}, but we have also observed that urea, carbon dioxide and trace amounts

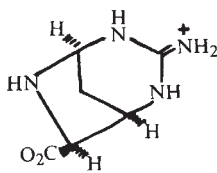


- (I) $R_1 = R_2 = R_3 = \text{OH}$ (viomycin)
 (II) $R_1 = R_2 = \text{H}$; $R_3 = \text{NH}_2$ (capreomycin IB)
 (III) $R_1 = \text{H}$; $R_2 = \text{OH}$; $R_3 = \text{NH}_2$ (capreomycin IA)

of glycine are formed. The basic amino-acid capreomycinine was originally assigned the gross structure (IV, stereochemistry not defined) on the basis of its physical and spectral properties⁴. We have confirmed this assignment by a total synthesis and established the relative and absolute chirality (IV) by a chemical correlation with viomycinine⁵ (V).



(IV)



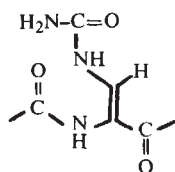
(V)

Capreomycin IB exhibits the same characteristic ultraviolet spectrum and low field signal in the nuclear magnetic resonance spectrum as that observed with viomycin (Table 1). It therefore seemed probable at an early stage that the same chromophoric unit (VI) was present in both antibiotics.

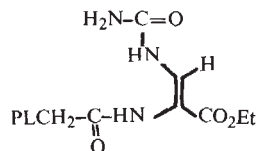
Hydrolysis of capreomycin with 0.1 M HCl results in the liberation of one equivalent of urea with the concomitant disappearance of the ultraviolet absorption and the low field proton. The first order rate constant for the loss of the chromophore is $2.28 \times 10^{-2} \text{ min}^{-1}$ (compared with viomycin $2.45 \times 10^{-2} \text{ min}^{-1}$) and this was shown, within the bands of experimental error, to be the same as the rate constant for the production of urea, after a correction had been made for the further breakdown of urea in the conditions of the reaction. The resultant desureacapreomycin gives a deep red ferric test and it readily reduces Tillman's reagent, and silver nitrate and copper acetate solutions. Although lacking ultraviolet absorption in acidic media, it has a strong maximum at 272 nm in basic solution. Desureacapreomycin is reconverted smoothly to capreomycin in the presence of excess urea and dilute acid. This establishes that no deep seated re-arrangement occurs during hydrolysis, and the reaction provides an extremely simple route to a series of modified capreomycin derivatives containing substituted ureas (B. W. B., A. H.-W., A. W. J., J. D. Hardstone and N. R. Panes, unpublished results). Oxidation of capreomycin with potassium permanganate yields formylurea and oxalic acid, although catalytic reduction gives a tetrahydro derivative possessing no chromophore, and which on total acid hydrolysis yields the amino-acids, diaminopropionic acid, alanine, β -lysine, and capreomycinine (ratio 2 : 2 : 1 : 1, respectively) but no traces of glycine. The extra mole of alanine, as it is not derived from any of the amino-acids present in capreomycin, must have been formed by reduction of the chromophoric unit.

This evidence confirmed that the chromophore (VI), that is, a ureide of dehydroserine, is present in both capreomycin and viomycin and the close similarity in chemical, physical, and spectral properties of the penaldete ureide⁶ (VII) (Table 1) with those of the two antibiotics has substantiated these proposals.

The question of possible geometrical isomerism about the ureide double bond has not yet been fully resolved. As only



(VI)



(VII)

Table 1 Electronic Spectra of Viomycin, Capreomycin IB, and a Synthetic Compound (VII) containing a Related Chromophore

Solvent	Viomycin	nm (ϵ) Capreomycin IB	Compound (VII)
H ₂ O	268 (24,000)	268 (24,040)	266 (22,100)
NaOH (0.1 M)	285 (15,000)	285 (15,040)	308 (24,000)

one isomer is formed in the syntheses of capreomycin and viomycin from the corresponding desurea derivatives, however, both antibiotics can be assumed to contain the most thermodynamically stable isomer. This agrees with the proposed structure (VI) for the chromophore and further evidence supporting this formulation will be reported later.

The sequence of the amino-acids in capreomycin IB has been determined from the evidence of end-group analyses of capreomycin itself and of the structure of the dipeptides obtained from partial acid hydrolyses. Capreomycin IB forms a *tri*-2,4-dinitrophenyl derivative which, on acid hydrolysis, yields *bis*-2,4-dinitrophenyl- β -lysine and β -mono-2,4-dinitrophenyl- $\alpha\beta$ -diaminopropionic acid. Hydrazinolysis of capreomycin indicated no free carboxyl groups, a conclusion in agreement with potentiometric titration data. The initial work on the antibiotic had established that partial hydrolysis in concentrated hydrochloric acid at 70° C yielded a number of peptides, of which four dipeptides were isolated and characterized², namely ala, β -lys, β -lys-dapr, dapr, cap and cap-ala. As we have established that both amino-groups of the β -lysine residue are free in the intact antibiotic, it seems likely that the alanyl- β -lysine dipeptide is an artefact; furthermore, we have not observed this dipeptide in our partial hydrolysates of capreomycin. We have isolated all the other dipeptides listed and have confirmed the previous structural assignments. The location of the chromophoric unit follows from the fact that further mild hydrolysis (0.1 M HCl) of desureacapreomycin results in the breakdown of the dehydroserine residue and in the release of a free carboxyl group of a diaminopropionic acid unit.

This evidence is completely consistent with structure (II) for capreomycin IB and the assignment satisfies the physical and spectral properties of the antibiotic. Although investigations on the other components of the complex are still in progress, it seems likely that capreomycin IA possesses structure (III) and capreomycins IIA and IIB correspond to the structures of capreomycins IA and IB except that they lack the β -lysine residues, and may even be artefacts. It is now apparent that viomycin and the capreomycins are members of a closely related family of antibiotics containing a dehydroserine ureide unit and a cyclized arginine system. The significance of these units in relation to the frequent occurrence of dehydroamino-acids and D-amino-acids in microbial peptides has been discussed elsewhere⁷. A recent addition to the viomycin series is tuberactinomycin⁸ which yields viomycinine (V) rather than capreomycinine (VI) on hydrolysis. From the structural information provided so far, it could differ from viomycin only in that a γ -hydroxy- β -lysine replaces the β -lysine fragment.

Received January 12, 1971.

¹ Bycroft, B. W., Cameron, D., Croft, L. R., Hassanali-Walji, A., Johnson, A. W., and Webb, T., *Experientia* (in the press).

² Herr, E. B., Haney, M. E., Pittenger, G. E., and Higgins, C. E., *Proc. Indiana Acad. Sci.*, **69**, 134 (1960).

³ Herr, E. B., and Redstone, M. O., *NY Acad. Sci.*, **135**, 940 (1966).

⁴ Herr, E. B., *Antimicrobial Agents and Chemotherapy*, 201 (1962).

⁵ Bycroft, B. W., Cameron, D., Croft, L. R., and Johnson, A. W., *Chem. Commun.*, 1151 (1968).

⁶ Bycroft, B. W., Cameron, D., Hassanali-Walji, A., and Johnson, A. W., *Tetrahedron Lett.*, 2539 (1969).

⁷ Bycroft, B. W., *Nature*, **224**, 595 (1969); *Proc. Tenth Europ. Peptide Symp.*, Abano, Italy (in the press, 1969).

⁸ Nagata, A., Ando, T., Izumi, R., Sakakibara, H., Take, T., Hayano, K., and Abe, J., *J. Antibiotics*, **21**, 681 (1968); Wakamiya, T., Shiba, T., Kanedo, T., Sakakibara, H., Take, T., and Abe, J., *Tetrahedron Lett.*, 3497 (1970).

LETTERS TO NATURE

PHYSICAL SCIENCES

White-light Three Dimensional Microscopy using Multiple-image Storing and Decoding

THERE is a need in microscopy for high quality imaging of transparent specimens which have a large axial depth compared with the depth of focus. Because of the necessarily limited axial depth of focus of conventional microscopes, when a biologist needs detailed information throughout the thickness of a specimen, without compromising lateral resolution, he has to take a series of successive photographs on separate plates (or film frames).

The problem of three dimensional microscopy has received considerable attention. Among the most recent methods, so called deep field microscopy^{1,2} consists of illuminating the specimen with a thin "sheet" of light, perpendicular to the optical axis of the microscope, and recording the topological sections successively, in the same photograph, with suitable re-focusing. This method does not seem to be readily applicable to transparent specimens. Considerably greater hopes, in view of biological applications, arose recently for the use of three dimensional coherent (laser) light holographic imaging. Unfortunately, no images of a quality even closely comparable with that of conventional microscopy have been obtained in holographic microscopy so far, using middle or high magnifications (which are required for high-resolution imaging). Indeed, it has now become clear that two severe restrictions result from the use of coherent laser light, needed to record holographic

images. The first is that the use of collimated laser light is equivalent to the use of an infinitely small condenser numerical aperture, with perfectly monochromatic light. This condition is generally considered to be unacceptable in microscopy. Moreover, the very high degree of coherence (spectral and especially spatial)^{3,4} generates very strong interference patterns because of the multiple scattering throughout the volume of the specimen. To increase the numerical aperture of the condenser, the use of "diffusers" has been established as the most suitable technique. Unfortunately, the introduction of diffusers in coherent illuminating beams also results in unacceptable image degradation, because of spurious diffraction and interference phenomena⁵. Here, we describe a method which seems to overcome these difficulties.

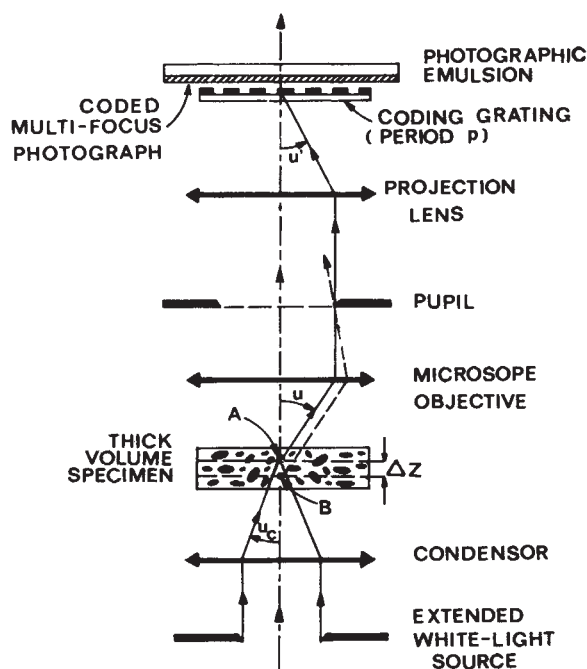


Fig. 1 Photographic microscope for multi-focus image recording.

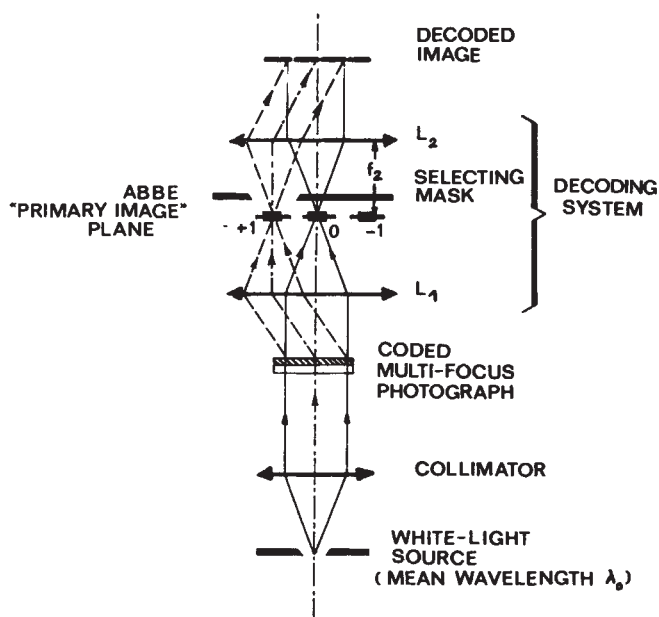


Fig. 2 Decoding system for extracting selected single-focus images.

The principle of our method can be understood with reference to a conventional photographic microscope (Fig. 1). A fine square-wave amplitude (black-and-white bars) grating is placed in front of the photographic emulsion. After processing, the exposed, coded photograph is replaced in its recording position. The specimen is removed, and the photograph illuminated with the microscope in its original recording arrangement, now described as "collimator", as in Fig. 2. A lens L_1 is placed after the photograph. In the focal plane of L_1 a coloured spectrum is produced, consisting of a series of elliptical spots, with the brightest at the centre. A second lens, L_2 , identical to L_1 , is placed with its front focus in the spectrum plane. In the rear focal plane of L_2 , an image of the microphotograph is seen, modulated by the coding grating. The spectrum plane was named "primary image" by Abbe⁶, who pointed out that it contained all the imaging information about the object. Abbe further showed that substantial changes in the final image are produced by occulting some parts of the "primary image" with the aid of a suitable mask. For example, if only the

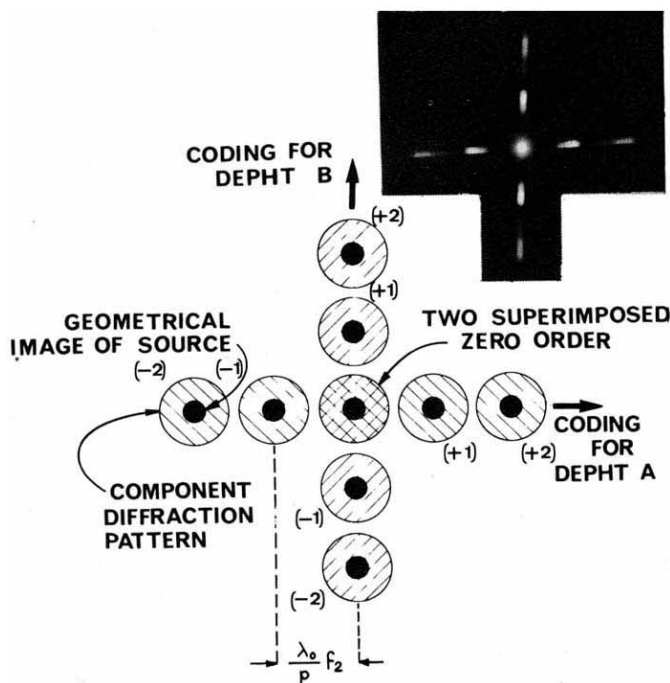


Fig. 3 Spectral diffraction patterns in Abbe "primary-image" plane of Fig. 2. Photographic inset shows spectrum for case of Fig. 6.

central diffraction pattern is isolated, the grating modulation disappears in the image formed by L_2 . Moreover, the final image is identical (in intensity) to that which would have been recorded directly without the grating, provided only that the "side-band" diffraction patterns are sufficiently separated from the central pattern, in the primary image plane.

It is interesting that the same unmodulated final image is obtained by using one of the side-band diffraction patterns, although the brightness is somewhat less. The extension of these basic concepts to the solution of "deep-field" microscopy is equally straightforward. For simplicity of the discussion, we consider that a "deep" volume object consists of optically separable "slices", each of which is considered as a separate specimen, when operating in incoherent illumination.

After exposing the photographic emulsion to the image of the first specimen, through the coding grating, we expose the same emulsion to the image of the second specimen, after rotating the grating into a new position; for example, by 90° . The processed, doubly exposed "coded" photograph is placed into the "decoding" arrangement of Fig. 2, which uses a small white light source to produce an almost collimated illuminating beam. The spectrum observed in the Abbe primary image plane is now more complicated than in the case of a single image (Fig. 3). In addition to the central pattern, there are now two orthogonal (90°) lateral side-band spectral patterns, each corresponding to one of the two images (described as *A* and *B*). One of the images may be made to appear, unmodulated, in the final image plane of L_2 by selecting any one of the single side-band patterns in the primary image plane corresponding to that image.

The final "decoded" images are not affected in any way by the intermediary use of the coding grating (a detailed theoretical analysis of our work will be published later). Each of the band-limited side-band spectral patterns contains all the imaging information. It is therefore sufficient to isolate any one of the side-band spectra, in order to produce a non-degraded image of the microphotograph in the focal plane of L_2 . A single image may be similarly selected in the case when a number of coded photographs are superimposed in the same emulsion, provided that each was coded with the grating in a different azimuthal orientation, and that only the correspond-

ing side-band pattern is used for imaging. The situation described has been well known, or may be readily deduced from a great number of publications dating back to 1873. Most recently, the method was singled out as particularly valuable for macroscopic applications; for example, alphanumeric information storage. In these cases, coherent light sources were considered to be the ideal decoding sources⁷. In high resolution microscopy, extended white light sources are, however, necessary. It may indeed be shown⁸ that the carrier-frequency photography principle is also applicable to diffraction-limited imagery in the strictest sense, notably in microscopy, in cases where considerable attention needs to be paid to the conditions in which diffraction-limited imaging information can be separated without loss in the primary Abbe spectral image plane. Perfect imaging may thus be achieved, as we demonstrate here.

We now return to the problem of three dimensional deep-field microscopy. We need only to note that a simple extension of the preceding discussions permits us to conclude that it is possible to record separately in a single emulsion, coded, axially separated images of a same volume microscopic specimen, the thickness of which is large or very large compared with the depth of field of the microscope. It thus becomes possible to store, in the plate, microscopic information about the specimen in such a way that it becomes possible to extract from a single photograph information about selected planes, along the specimen's axial depth, while at the same time conserving the resolution and contrast of each of the selected, axially separated images.

The number of superimposed, storable, coded images is limited. In the reconstruction, one may extract either a single image, with a selected depth coordinate, or several images simultaneously. The latter offers the very interesting possibility of determining and of precisely measuring three dimensional angular orientations throughout the volume.

An extension of this possibility to three dimensional angular measurements in high-energy particle physics may be of interest. The advantage of our method, compared with conventional microscopy, for 3-D angular orientation measurements throughout a volume results from the fact that our method permits one to "freeze" in a single photographic plate, without any loss of the maximum possible attainable optical resolution, the image information about the entire three dimensional volume of interest. Thus our method, even though seemingly more complicated at the recording stage, not only permits one to surmount very great difficulties which characterize conventional microscopy, with regard to photographic distortion, between many different photos, but it also eliminates the need

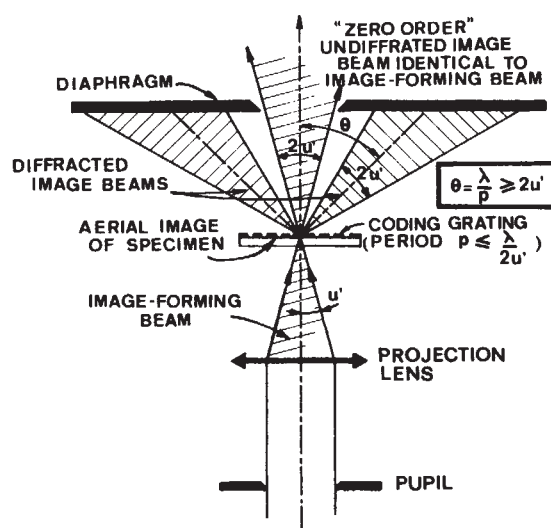


Fig. 4 Auxiliary diagram for theoretical discussion in text.

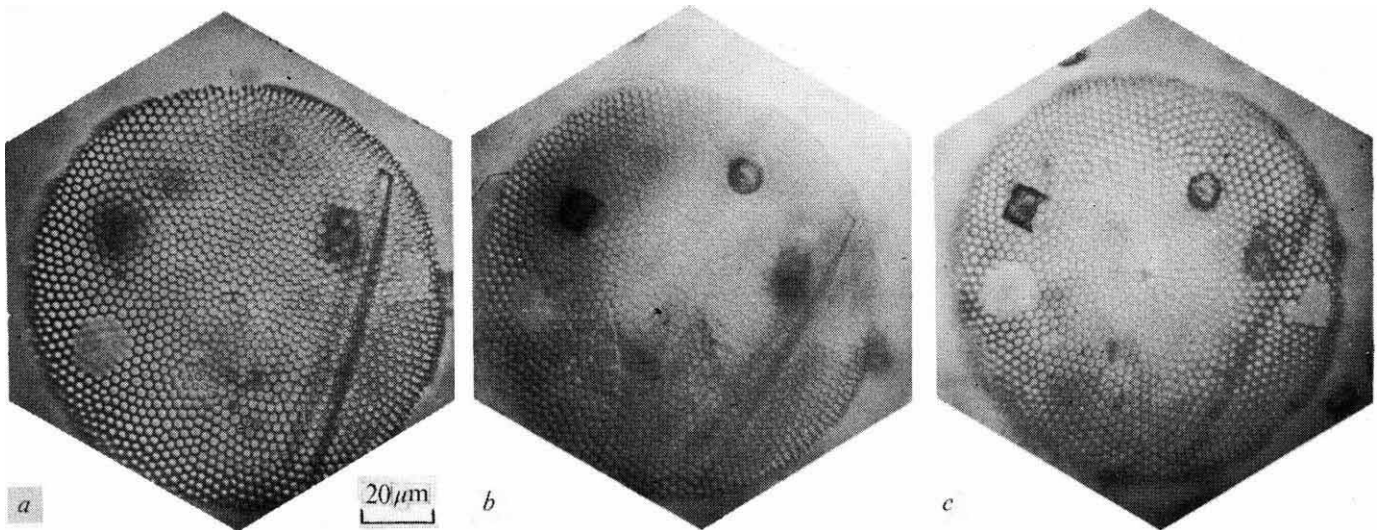


Fig. 5 Micrographs of a diatom ($\times 28$; numerical aperture, 0.75 objective, see text). *a*, Direct white light image of 0 μm depth section. *b*, Superposition of four coded images using arrangement of Fig. 1 and grating with period $p=8 \mu\text{m}$. *c*, Superposition of same four images as in (*b*), by accumulation, with successive focusing only, without coding, shown for comparison.

for exacting alignment requirements, between numerous separate micrographs, which characterizes conventional microscopy in three dimensional volume measurement applications.

Finally, it will appear, from further theoretical discussion of the parameters used in our experimental verifications, that we did indeed fulfil the conditions required for non-degraded, diffraction-limited imaging, as indicated above. We show two sets of experiments for illustration: one with a small depth of focus (high axial resolution) $2\Delta z = 3.6 \mu\text{m}$ (Fig. 5), and the other with a very large depth of focus (low axial resolution) $2\Delta z = 80 \mu\text{m}$ (Fig. 6).

In one of the preliminary experiments, we used a photographic microscope with both low and high magnifications. The optics used were an objective $\times 40$, numerical aperture, 0.75 for the diatom of Fig. 5 and an objective $\times 6.3$, numerical aperture, 0.16 for the hydra of Fig. 6. A full numerical aperture illumination was used in both cases. The projection eyepieces were respectively $\times 8$ for the diatom and $\times 5$ for the hydra. The photographic camera had a magnification coefficient of $\times 0.52$ for the diatom and $\times 1.25$ for the hydra. The total magnification was thus $40 \times 8 \times 0.52 = 166$ for the case of the diatom and $6.3 \times 5 \times 1.25 = 39$ for the hydra. The numerical aperture u' in the final image space (Fig. 1) was therefore $0.75/166 = 0.45 \times 10^{-2}$ for the diatom and $0.16/39 = 0.41 \times 10^{-2}$ for the hydra. A coding square-wave "black-and-white bar" grating was placed immediately in front of the photographic emulsion. The period of the grating was chosen to be sufficiently fine for angularly separating the side-band spectral patterns from each other. This was accomplished with reference to the auxiliary schematic diagram of Fig. 4, which

deals only with the transmission of the aerial image through the coding grating itself (no photographic recording is involved here). As seen, the "zero-order", undiffracted-image beam will pass through the grating (assumed perfect⁸) without in any way being modulated or affected by the diffracted beams, provided only that the period p is less than $\lambda/2u'$, and that the diffracted beams are masked out by a suitable diaphragm, as shown. Indeed, in this case the side-band diffracted beams do not overlap the central "undiffracted" beam ($\theta \geq 2u'$). The condition for preservation of the quality of the aerial image, $p \leq \lambda/2u'$, is generally insufficient by itself. Additional conditions which must be taken into account are: (1) dimensions of the white light source used in the reconstruction; (2) spectral intensity distribution (spectrum); (3) the spectral sensitivity of the photographic plate; and (4) its processing conditions^{3,4}. In the case of illumination with a monochromatic point source, each of the spectral patterns would be a point, with the $+1$ (or -1) order pattern centred at $(\lambda_0/p)f_2$ from the zero order pattern, the $+2$ (or -2) centred at $2(\lambda_0/p)f_2$ from the centre, and so on, in the case when the plate contains only a grating, without any other image. In the case of a coded image, the spectral orders now consist of diffraction patterns of diameter $2u'f_2$, where u' is the numerical aperture in the image space. When the source itself is extended, so that its image in the Abbe plane has a diameter $2\theta_s f_2$ (where θ_s is the angular diameter of the source image), each point of this image is now surrounded by the diffraction patterns of diameter $2u'f_2$, so that the total extent of the diffraction pattern is the sum $2\theta_s f_2 + 2u'f_2$. For a source of extended spectral intensity, each spectral component (of wavelength λ) gives a



Fig. 6 Micrographs of a hydra ($\times 6.3$; numerical aperture, 0.16 objective), using Nomarski differential interference contrast. *a*, Superposition of two coded images, using $p=8 \mu\text{m}$ grating, with two 90° orientations (see spectrum in Fig. 3). (*b*) and (*c*) Decoded images, 80 μm apart, extracted from (*a*) using arrangement of Fig. 2. Slicing resolution $2\Delta z = 80 \mu\text{m}$.

single point image, at $(\lambda/p)f_2$ from the centre, in the case of point-source illumination, using the grating alone. Accordingly, when using the coded image and an extended white light source, each of the single spectral points is now surrounded by the diffraction patterns $2u'f_2$, themselves centred on the extended source image $2\theta_s f_2$. As a result, the $(2\theta_s f_2 + 2u'f_2)$ pattern is elliptically enlarged in the spectral dispersion direction⁹. In practice, these several multiple convolutions are quite simply determined, in a good first approximation, by a visual (or photographic) examination of the Abbe spectrum of a test object, such as the spectrum which appears in the inset of Fig. 3. It is only necessary to make certain that the $+1$ (or -1) order patterns are well separated from the other orders. This "first order isolation test" also takes into account the photographic recording and processing conditions of the original image, which are otherwise more difficult to describe. In practice a microscope may be equipped with a simple table indicating the grating period p which is to be used for a given objective and source. The photographic conditions may also be met simply in practice. Two classical conditions are theoretically and practically very suitable. The first, which we used in the case of the diatom (Fig. 5) is the well known Gabor $\gamma_N \gamma_P = 2$ condition [2], where γ_N is the slope in the linear range of the characteristic H-D photographic curve of the original coded image (negative N), and γ_P the slope of a positive copy of it. The second is even simpler. The original negative itself may be used: (a) in case of the usual low contrast specimens, with no restriction on the gamma; (b) in cases of arbitrarily high contrast objects, provided only that the image is processed with a moderately low gamma, say, up to 2.5. This method was used in the case of the hydra. Recording in the photographic range above the threshold is achieved by suitable pre-exposure in both cases. Suitably fine-grain emulsions need to be used to record a good contact image of the grating. In our preliminary experiments we used Agfa-Gevaert Scientia 10E56 plates.

A review of image encoding in modulated gratings from 1899 to 1970 is given in ref. 10.

We acknowledge the support of the National Science Foundation. We also acknowledge useful conversations with Professor Dennis Gabor, and Maurice Halioua and François Poisson.

Massachusetts General Hospital,
Harvard Medical School,
and Shriners Burns Institute, Boston

State University of New York,
Stony Brook

Institut d'Optique,
Paris

State University of New York,
Stony Brook,
and Harvard Medical School

Received November 13, 1970; revised April 29, 1971.

¹ Simon, W., *Rev. Sci. Instrum.*, **36**, 1654 (1965).

² McLachlan, jun., D., *Sci. Res.*, **3**, 65 (1968).

³ Maréchal, A., and Françon, M., *Diffraction, Structure des Images* (Revue d'Optique, Paris, 1960).

⁴ Stroke, G. W., *An Introduction to Coherent Optics and Holography*, second ed. (Academic Press, New York and London, 1969).

⁵ Gabor, D., *IBM J. Res. Develop.*, **14**, 509 (1970).

⁶ Allen, R. D., David, G. B., and Nomarski, G., *Z. Wissenschaft. Mikroskopie Mikroskop. Technik.*, **69**, 193 (1969).

⁷ Bestenreiner, F., Deml, R., and Greis, U., *Photographic Science and Engineering*, **14**, 1 (1970).

⁸ Stroke, G. W., *Handbuch der Physik*. (edit. by Flugge, S.), **XIX**, 426 (Springer, Berlin and Heidelberg, 1967).

⁹ Stroke, G. W., Furrer, F., and Lamberty, D. R., *Optics Commun.*, **1**, 141 (1969).

¹⁰ Biedermann, K., *Optica Acta*, **17**, 631 (1970).

Asymmetry of the Internal Seiche in Loch Ness

LOCH NESS seems to be particularly suitable for internal wave research. Because it is freshwater the effects of salinity, which are so difficult to monitor in the ocean, are absent. The wind has a strongly preferred direction up and down the length of the steep-sided Loch, which is of a fairly regular shape (Fig. 1) and great depth, and which supports a well developed thermocline and rich microstructure¹ in the late summer. Long range effects and mean drifts (other than those associated with the rivers and streams which enter or leave the Loch) which occur in the ocean are absent, and so the Loch provides an excellent open air laboratory for testing theories of internal wave generation, propagation and decay.

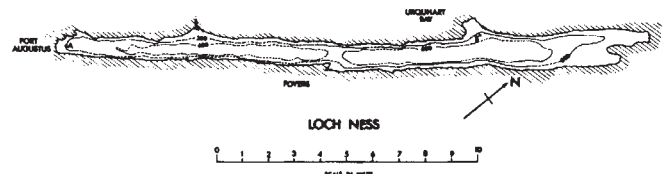


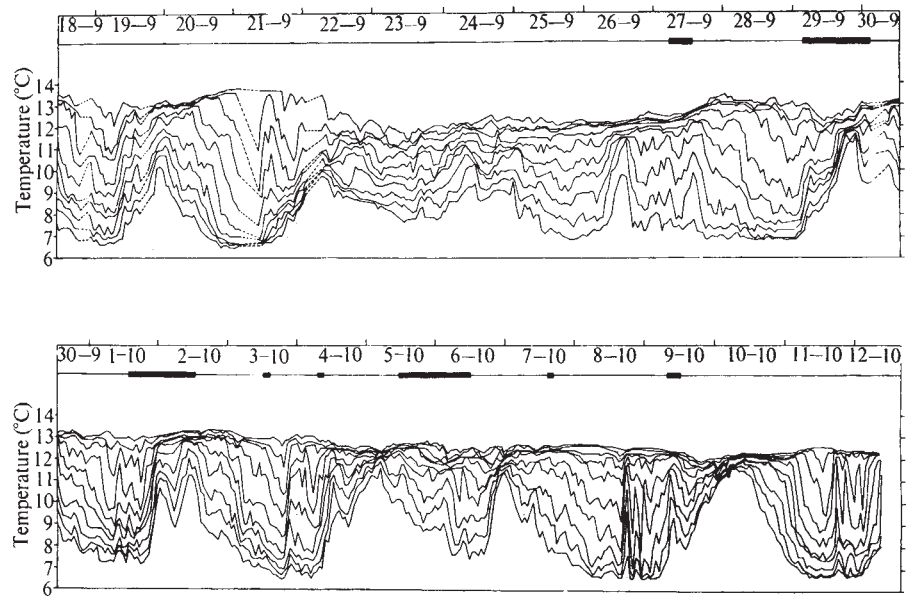
Fig. 1 Loch Ness. The depth contours are marked in feet.

The internal seiches of Loch Ness were first studied early this century^{2,3}. The dominant internal seiche has a wavelength twice the length of the Loch, and a marked asymmetry. In 1952 Mortimer⁴ used a chain of thermistors to measure the temperature of the Loch at nine levels down to 100 m near Fort Augustus (A, Fig. 1). The main longitudinal internal seiche is characterized by a relatively sudden rise in temperature followed by a less rapid rise and a rather gradual decrease before the next sudden rise. (The profiles in many ways resemble those of the surface elevation of a river in which there is a tidal bore.) During the period of observation from mid-August to early October the seiche had a period of rather more than 2 days and the sudden rise in temperature occurred in about 4 h and accounted for most of the temperature increase. The asymmetry cannot be accounted for by the presence of the thermocline and associated microstructure, for although this leads to sudden changes in temperature at some levels (as the edge of the thermocline moves past a thermistor) a symmetrical wave would produce similar changes as it rises and falls. Nor do the abrupt changes in temperature result from a tendency for the seiche to steepen and break at the gradually shoaling end of the Loch, as the increase in temperature associated with the changes implies a depression of the isotherms, and a rise in their level is expected as an internal wave approaches a beach^{5,6}. The asymmetry does not seem to depend on the direction of the prevailing wind. An examination of the wind measurements indicates that the seiches are forced chiefly by the wind. There are occasional periods (for example, September 26 to October 5, 1952) when the wind is probably insufficient to influence the seiche, and the asymmetry continues during these times of almost free oscillation.

Observations of the temperature structure of the Loch, using thermistors at ten levels at a station in Urquhart Bay (B, Fig. 1), were made from September 7 to October 12, 1970, and these confirm the asymmetrical shape of the seiche (Fig. 2).

It is suggested that non-linear effects are the main cause of the observed asymmetry. As a simplification we consider a model consisting of two layers, the upper of density ρ_1 , and mean depth h_1 , and the lower of greater density ρ_2 and mean depth h_2 , contained in a rectangular tank of length l and bounded above and below by fixed horizontal planes. Previous analysis (ref. 7, particularly equation (A1.2)) of the main longitudinal internal seiche similar to that developed by

Fig. 2 The variation of temperature (measured in °C) recorded by ten thermistors spaced at 3 m intervals between 29 m and 56 m below the surface of Loch Ness at position B (see Fig. 1) from September 18 to October 12, 1970. The horizontal scale is marked in days and below this are marked the periods of strong south-west winds (when the wind exceeded 4 m s^{-1} at an anemometer 1 m above the water surface at Temple Pier in Urquhart Bay). No strong north-east winds blew during the period. Temperatures are marked at hourly intervals.



Penney and Price⁸ for surface standing waves is valid when an expansion parameter

$$\delta = \frac{3al^2|\rho_1 h_2^2 - \rho_2 h_1^2|}{8\pi^2 h_1^2 h_2^2 (\rho_1 h_1 + \rho_2 h_2)}$$

is much less than unity. Here a is the half amplitude of the seiche motion of wavelength $2l$ and the density difference between the two fluids is supposed to be small. Introducing values appropriate to Loch Ness in September ($h_1=45 \text{ m}$, $h_2=105 \text{ m}$, $l=35 \text{ km}$, temperature of epilimnion= 11.8° C , temperature of hypolimnion= 5.8° C) we find that $\delta=126a$ approximately, with a measured in metres. For a seiche of amplitude $a=4 \text{ m}$, which is typical, δ is much greater than unity. The analysis previously used is thus invalid and a non-linear long wave approximation is appropriate. In these circumstances waves have a tendency to steepen and to form surges or bores. A simple analysis following that of Chester⁹ for surface waves but neglecting diffusion and dissipation shows that if a simple harmonic body force $A \cos \sigma t$ is applied to the upper layer (thus modelling the wind-induced stress at the surface of the Loch) the response of the interface at one end of the tank is given by

$$\eta = \frac{2h_1 h_2 (\rho_1 h_2 + \rho_2 h_1)}{\rho_2 h_1^2 - \rho_1 h_2^2} f(t) \quad (1)$$

where $f(t)$ is a solution of the equation

$$\left(\varepsilon^{-1} f - \frac{2r}{\pi}\right)^2 = d + \frac{1}{2} (\sin q) \cos \sigma t \quad (2)$$

with the constant d chosen so that f has zero mean, and

$$q = \frac{\rho_1 A (\rho_1 h_2^2 - \rho_2 h_1^2)}{(\rho_1 h_2 + \rho_2 h_1)^2 \sigma^2}, \quad \varepsilon = \frac{4|q| \left(1 - \cos \frac{\sigma l}{c}\right)}{3l \left|\cos \frac{\sigma l}{c}\right|}$$

$$r = \frac{\pi c}{3l \sigma \varepsilon^{\frac{1}{2}}} \tan \frac{\sigma l}{c}$$

with
$$c^2 = \frac{gh_1 h_2 (\rho_2 - \rho_1)}{\rho_1 h_2 + \rho_2 h_1}$$

The solutions of (2) are discontinuous⁹ if $|r| < 1$, for example, when the forcing frequency is close to the natural frequency, $(\pi c/l)$, of the main seiche, and then represent surges or shock waves. The solutions (1) have a singularity (and become invalid) when $\varepsilon=0$, that is when $\gamma = \frac{\rho_1 h_2^2}{\rho_2 h_1^2} = 1$. It can be shown¹⁰

that if the forms of the surge are restricted to those in which there is an energy loss (rather than a gain) as must occur in practice, then the surge will be a rise in level of the interface if $\gamma < 1$ or a fall in level if $\gamma > 1$. The latter case applies to Loch Ness. The amplitude of the discontinuity varies as $|\gamma - 1|^{-1/2}$ and increases as ε decreases.

Laboratory experiments have demonstrated the nature of the motion associated with the presence of the internal surge. A horizontal tube with internal dimensions 487.5 cm in length, 10 cm in height and 10.25 cm in width is completely filled with two layers of water and paraffin (US kerosene, specific gravity 0.78). The effect of rocking the tube through a small angle, 2α (28' in the experiments), about a horizontal axis is equivalent to applying a small periodic horizontal body force of amplitude ga to the fluid. ((1) is valid for such a distributed body-force if $\rho_1 A$ is taken equal to $ga(\rho_2 - \rho_1)$.) When the tube is rocked at frequencies very close to the natural frequency of the main seiche a surge is observed to grow rapidly during the first one or two oscillations. The form of the front of this surge is shown in Fig. 3. Only part of the tube is shown, half way between the centre and the right hand end, and the interface in the remainder is fairly flat. The values of γ in the various experiments are given in the caption. In all the photographs the front associated with the surge is moving to the right, and it is seen to reflect from the plane end of the tube and travel back with little overall change of form. With a shallow lower layer ($\gamma < 1$; a, c, e) the level is raised after the passage of the surge, whereas if the upper layer is the shallower ($\gamma > 1$;

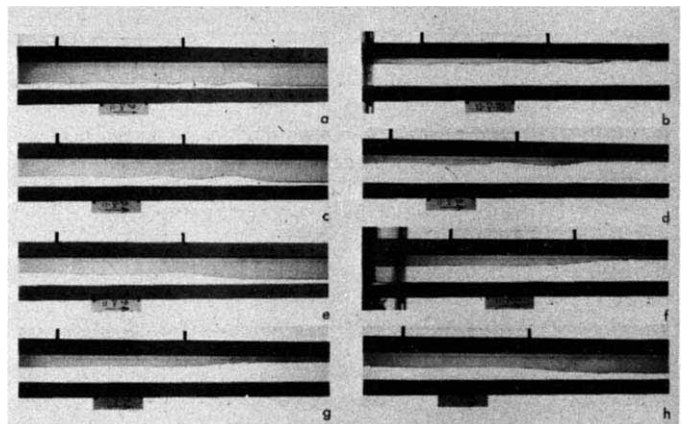


Fig. 3 The front of an internal surge: (a) $\gamma=0.05$; (b) $\gamma=16.1$; (c) $\gamma=0.14$; (d) $\gamma=5.15$; (e) $\gamma=0.34$; (f) $\gamma=1.91$; (g) and (h) $\gamma=0.81$.

b, d, f) the level is depressed. When the fluids are of almost equal depth (*g, h*) the surge contains two waves, one of elevation and one of depression 180° out of phase, which resemble waves found in deep layers in the earlier study⁷. The amplitude of the jump in levels increases as the depths become more nearly equal ($\gamma \rightarrow 1$) as predicted. The surge has the form of an undular bore with a train of waves moving with the surge front, and reflecting at the ends of the tank. The presence of surface tension at the water-paraffin interface seems to damp out some effects in this wave train. Although the qualitative effects were similar to those observed for the immiscible fluids, as many as twenty-five short waves were seen to form in the undular bore in an experiment made with miscible fluids, brine and water, and instabilities in the shear flow both ahead and behind the front¹¹ and a degradation of the wave train possibly resulting from resonant interactions¹² were noticed. A similar surge is found to develop if the tube is brought into a horizontal position from a slightly tilted position of rest; this experiment models the development of a surge in a lake following the relaxation of a wind stress. The front of the surge develops in about three-quarters of the natural period of oscillation.

The asymmetry of the internal seiche of Loch Ness thus seems to be the result of a tendency for internal surges to develop in a fashion similar to that simulated in the experiments as a result of non-linear long wave effects. It is likely that the effect of the Earth's rotation is principally to cause a small alternating transverse tilt of the isotherms across the Loch during the seiching motion.

It seemed possible that the presence of internal undular bores or surges in Loch Ness associated with the asymmetrical profiles of temperature were undetected by Mortimer⁴ as he only recorded temperature at intervals of 18.2 min, and the recent more continuous observations were made to see if this was so. The record shown in Fig. 2 is punctuated by sudden increases of temperature followed by gradual falls, but unlike the Fort Augustus record⁴, the rise is frequently seen to occur in two parts. The first is often accompanied by waves of period about 50 min and it precedes the second rise by about 14 h (approximately the time taken for the surge to travel to and from the north-east end of the Loch). The waves cannot definitely be associated with the progressing structure of the surge front, as it is possible that they result from an interaction of the front with the topography of Urquhart Bay and the consequent production of a transverse oscillation*. Another possibility is that the waves are lee waves produced by the flow of the seiche-induced currents at the sill near Foyers (Fig. 1) and are similar in form to the internal bores found in the Straits of Gibraltar¹³. Weak oscillations were recorded down to periods of 10 min (the most rapid which could be resolved by the thermistors). The result of this investigation will be described in more detail later, and we hope to continue the observations of internal waves and currents in Loch Ness next year.

Since this communication was started Dr Mortimer has told me of his unpublished observations made using two thermistor chains near Foyers. These clearly show internal motions of period half those observed at the same time at Fort Augustus. The rapid rise in temperature measured at Fort Augustus is again seen in the Foyers record and the rise at Fort Augustus occurs half-way between alternate rises at Foyers, showing that the surge travels up and down the Loch and reflects from the ends as found in the laboratory experiments. The simple interpretation of the temperature oscillation in terms of a simple uninoded internal seiche proposed by Watson² and Wedderburn³ thus seems to be inadequate.

* Dr K. Hunkins of the Lamont-Doherty Geological Observatory has shown me the results of some of his observations in Seneca Lake in New York State which indicate the presence of an internal undular surge with waves of about 15 min period associated with a fall in the isotherm level and similar to that found in the laboratory experiments (Fig. 3b).

I thank Professor J. Darbyshire for allowing the use of the thermistor chain and Mr A. Edwards for calibrating and helping to install it. Power, accommodation and boats were supplied by Dr Bob Love of the Loch Ness Investigation Bureau whose help and interest I gratefully acknowledge.

S. A. THORPE

*National Institute of Oceanography,
Wormley, Godalming,
Surrey*

Received December 9, 1970.

- ¹ Simpson, J. H., and Woods, J. D., *Nature*, **226**, 832 (1970).
- ² Watson, E. R., *Geog. J.*, **24**, 430 (1904).
- ³ Wedderburn, E. M., *Trans. Roy. Soc. Edin.*, **45**, 407 (1907).
- ⁴ Mortimer, C. H., *Proc. Intern. Assoc. Appl. Limnol.*, **12**, 66 (1955).
- ⁵ Cairns, J. L., *J. Geophys. Res.*, **72**, 3563 (1967).
- ⁶ Thorpe, S. A., thesis, Univ. Cambridge (1966).
- ⁷ Thorpe, S. A., *J. Fluid Mech.*, **32**, 489 (1968).
- ⁸ Penney, W. G., and Price, A. T., *Phil. Trans. Roy. Soc., A*, **244**, 254 (1952).
- ⁹ Chester, W., *Proc. Roy. Soc., A*, **306**, 5 (1968).
- ¹⁰ Benjamin, T., and Brooke, J., *Fluid Mech.*, **25**, 241 (1966).
- ¹¹ Thorpe, S. A., *J. Fluid Mech.*, **32**, 693 (1968).
- ¹² Davis, R. E., and Acrivos, A., *J. Fluid Mech.*, **30**, 723 (1967).
- ¹³ Zeigenbein, J., *Deep-Sea Res.*, **16**, 479 (1969).

Isolation and Identification of Sterols from a Pleistocene Sediment

BECAUSE of their chemical stability, alkanes and fatty acids have been widely used as indicators of biological origin in studies of sediments¹⁻⁴, but their formation may often be attributable to abiogenic syntheses and transformations⁵ so that their distribution patterns should be approached with caution. Little effort has been expended on analysis of sediments for relatively unaltered molecules which are biologically more significant, such as sterols which are present in sea water⁶, bottom muds (both marine and non-marine)^{7,8}, surface soils⁹, and an Eocene shale¹⁰. If such molecules are present in sediments representing a wide period of geological time, their significance with regard to depositional environment and contributory organisms would exceed any other biological marker in common use. We report some organic geochemical studies of a Pleistocene basin and a contemporary lake where we are attempting to correlate alkane, fatty acid, steroid, amino-acid and sugar distribution patterns with stratigraphy and biological environment.

Mono Lake is one of the strongly alkaline desert lakes of eastern California, and has a combination of high organic productivity with almost no scavengers. It is situated in a structural downwarp and has been continuously in existence for ~3 m.y. (refs. 11, 12). Sediments deposited in this lake show no clearly freshwater fauna but contain an abundance of algal debris, diatoms, ostracods and, locally, gastropods.

The sediment analysed contained alternate bands of algal silt and diatomite. It resides within a complete Pleistocene lacustrine sequence and its position within that sequence (that is, 80 m below the top of the Wilson Creek Formation, which is the latest Pleistocene sediment exposed in the Mono Basin) suggests an age ~130,000 yr.

We have isolated and identified the components of a complex mixture of sterols by gas-liquid chromatography (GLC) and combined gas chromatography-mass spectrometry (GCMS). Powdered sediment (9.95 g) was extracted using a mixture of benzene, acetone and methanol (2 : 1 : 1 v/v). The concentrated extract (6.75% w/w of sediment) was chromatographed on a silica gel column eluting successively with heptane, benzene and methanol. The methanol fraction was saponified with 5% NaOH and the non-saponifiables were dissolved in ether, chromatographed on a modified McCarthy-Duthie

column¹³ and the ether collected. The presence of sterols in this fraction was established on analytical thin-layer chromatographic plates (silica gel; 0.25 mm, using ceric sulphate and sulphuric acid spray reagent) by comparison with authentic sterols. Isolation of the sterol fraction was carried out by preparative thin-layer chromatography on silica gel with chloroform and ethyl acetate (3 : 1 v/v) as the mobile phase. The total fraction in pyridine solution was treated with *bis*-(trimethylsilyl) fluoroacetamide and trimethylchlorosilane (5 : 1 v/v). The resultant sterol trimethylsilyl ethers were analysed by GLC on a Perkin-Elmer Model 900 instrument using JXR, OV-17 and OV-210 as liquid phases. High and low voltage (70 and 16 eV) mass spectrometry (GEC-AEI MS-12) using a direct introduction solid probe yielded molecular ion data for the principal components of the complex mixtures. Single component identifications were achieved by GCMS using an Aerograph 204 gas chromatograph (flame ionization detector) coupled directly to a Dupont 492-1 mass spectrometer without a molecular separator or effluent splitting device.

Table 1 Identities of Sterols found in a Mono Lake Sediment

Peak	Retention index * (JXR)	Identity
1	3,120	
2	3,155	Cholesterol † TMS and cholestanol ‡ TMS
3	3,195	Brassicasterol † TMS
4	3,250	Campesterol † TMS
5	3,285	Stigmasterol † TMS and compound ‡ of molecular weight 486
6	3,340	Stigmasterol † TMS and β -sitosterol TMS †
7	3,410	Compound of molecular weight 502 thought to be lanosterol ‡ TMS

* GLC retention indices from a 10 foot $\times$ 1/8 inch stainless steel column containing 3% JXR on 60-80 mesh (gas Chrom Q) at 265° C.

† These identifications made by GLC and GCMS analyses.

‡ These identifications made by GCMS alone.

The results of the analysis of the purified sterol fraction in the form of trimethylsilyl ethers (TMS) by GLC is shown in Fig. 1. Comparison of the retention data obtained on two liquid phases (JXR, OV-17) with those of reference sterol derivatives suggested the following assignment of peak 2 to cholesterol TMS, peak 3 to brassicasterol TMS, peak 4 to campesterol TMS, peak 5 to stigmasterol TMS and peak 6 to β -sitosterol TMS. But analysis by GCMS indicated that some of the peaks contained more than one component. Peak 2 was shown to contain the TMS ethers of cholesterol and cholestanol, peak 5 contained a derivative of molecular weight 486 as well as stigmasterol TMS and peak 6 was chiefly stigmasterol TMS with a little β -sitosterol TMS. The assignments given to the chief peaks in Fig. 1 are also shown in Table 1. Further analyses by GLC on a more polar liquid phase (OV-210) confirmed the multicomponent nature of peaks 5 and 6 (Fig. 1).

The types of sterol found in the sediment examined strongly suggest that they had an algal origin and underwent a certain amount of reduction during sedimentation and the initial stages of lithification. Indeed, cholesterol, stigmasterol and β -sitosterol are known components of fresh water algae^{14,15} and cholesterol, brassicasterol, campesterol and β -sitosterol have been found in marine algae¹⁶. It may, therefore, not be possible to use sterol distribution patterns for identification of the deposition environment of a sediment (compare ref. 8).

Analyses of *in vitro* cultures of Mono Lake wild algae so far confirm the algal origin of the unsaturated sterols isolated from the sediment. There is no evidence for the presence of fully saturated sterols in the algae.

The identification of fully saturated counterparts of algal sterols, together with the unaltered compounds, is good evidence that at least partial reduction of naturally occurring

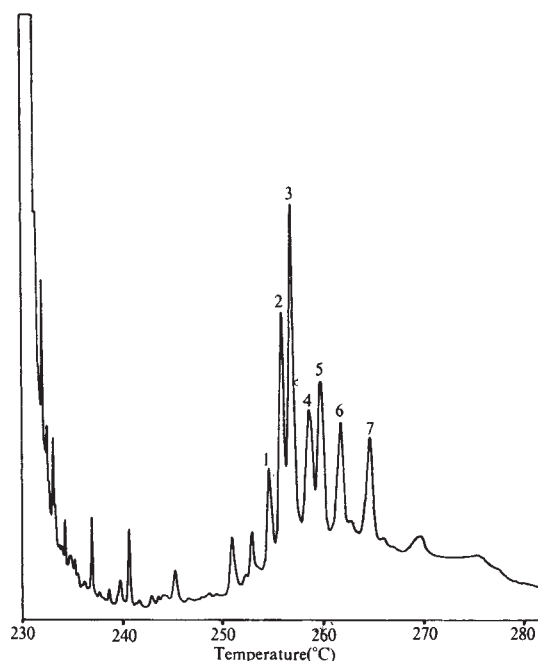


Fig. 1 Gas chromatogram of sterol trimethylsilyl ethers from a Mono Lake sediment obtained using a 10 foot $\times$ 1/8 inch stainless steel column packed with 3% JXR on 60-80 mesh ('Gas Chrom Q'). The column was programmed between 230° C and 280° C at 2° C min⁻¹.

molecules has taken place within the 130,000 yr since deposition. Simulated diagenesis experiments (using pressure, relatively mild temperatures and reducing conditions) seem to generate significant quantities of the fully reduced steroid hydrocarbons (steranes). Further work may not only allow chemotaxonomic correlations of organic compositions (alkanes, fatty acids, sterols, amino-acids and sugars) of sediments and contemporary organisms, but may also yield more specific information about the rates of the alteration of organic material in a geological environment and other parameters involved.

This work was supported by NASA.

WILLIAM HENDERSON
WALTER E. REED
GORDON STEEL
MELVIN CALVIN *

Department of Chemistry,
University of California,
Berkeley, California 94720

Received April 6, 1971.

* Present address: Laboratory of Chemical Biodynamics, University of California, Berkeley, California 94720.

- Eglinton, G., in *Advances in Organic Geochemistry* (edit. by Schenck, P. A., and Havenaar, I.), 1 (Pergamon Press, Oxford, 1968).
- Blumer, M., and Cooper, W. J., *Science*, **158**, 1463 (1967).
- Oró, J., Nooner, D. W., Zlatkis, A., Wikström, S. A., and Barghoorn, E. S., *Science*, **148**, 77 (1965).
- Henderson, W., Wollrab, V., and Eglinton, D., in *Advances in Organic Geochemistry* (edit. by Schenck, P. A., and Havenaar, I.), 181 (Pergamon Press, Oxford, 1968).
- Studier, M. H., Hayatsu, R., and Anders, E., *Geochim. Cosmochim. Acta*, **32**, 151 (1968).
- Matthews, W. S., and Smith, L. L., *Lipids*, **3**, 239 (1968).
- Schwendinger, R. B., and Erdman, J. G., *Science*, **144**, 1575 (1964).
- Attaway, D., and Parker, P. L., *Science*, **169**, 674 (1970).
- Meinschein, W. G., and Kenny, G. S., *Anal. Chem.*, **29**, 1153 (1957).
- Mattern, G., Albrecht, P., and Ourisson, G., *Chem. Commun.*, 1570 (1970).
- Gilbert, C. M., Christensen, M. N., Al-Rawi, Y., and Lajoie, K. R., in *Studies in Volcanology* (edit. by Coats, R. R., Hay, R. L., and Anderson, C. A.), Memoir 116 (Geological Society of America, 1968).

- ¹² Lajoie, K. R., thesis, Univ. California, Berkeley (1968).
¹³ McCarthy, R. D., and Duthie, A. H., *J. Lipid Res.*, **3**, 117 (1962).
¹⁴ Reitz, R. C., and Hamilton, J. G., *Biochem. Physiol.*, **25**, 401 (1968).
¹⁵ de Souza, N. J., and Nes, W. R., *Science*, **162**, 363 (1968).
¹⁶ Ikekawa, N., Morisaki, N., Tsuda, K., and Yoshida, T., *Steroids*, **12**, 41 (1968).

Nature of Quickclays

THE strength of clay soils *in situ* is often greater than the disturbed or remoulded strength and the ratio of these two values is a measure of the "sensitivity" of the soil; this may vary from ~ 1 for a London clay to $\sim 1,500$ for a Canadian Leda clay¹. The extremely sensitive soils, known as quickclays, are chiefly found in northern North America and Scandinavia, and they present a considerable geotechnical hazard because of a tendency to failure by landslide. The loss of strength at failure is remarkable—the material changes from a strong brittle soil into a viscous liquid, and this has proved difficult to explain.

The only substantial current theory is that of Rosenqvist² who proposed that the typical quickclay properties were produced by postdepositional leaching. This theory requires that the quickclay structure be formed by clay mineral particles in a "cardhouse" structure and that the cohesion in this system be reduced by the removal of soil water ions by fresh water leaching, the initial deposition being in sea water. The theory has been criticized³ and does not explain certain observations on Canadian quickclays⁴.

Rosenqvist, and most other investigators, have considered quickclays to be real clay soils and have expected the clay mineral content to determine the failure mechanism. Analyses of landslide soils^{5,6} show that clay minerals are not present in significant amounts and suggest that the other soil particles play the key role in determining quickclay properties. If a supply of fine non-clay mineral soil material is a requirement for quickclay formation then the geographical distribution is explicable. The abundant fine material formed by glacial action is incorporated into the quickclays.

There are essentially two types of interparticle bond in soils, long range active bonds and short range inactive bonds. The long range bonds exist between clay particles and depend on the charge of the particles, the presence of solute soil water ions and the polarized nature of water. The short range bonds exist between uncharged particles (for example, quartz silt particles) and depend on particle contact; once the contact is disturbed the bond is ineffective. The most essential requirement for quickclay formation is that short range bonds predominate in the soil system.

Leaching an ion rich soil weakens the clay-clay bonds and thereby effectively increases the proportion of short range bonds. Thus leaching can be seen as one factor contributing to quickness, but not the key factor. In fact a soil composed completely of clay minerals cannot be leached into a state of quickness³. The soil particles must also have a very low settling velocity in water and the system must have a high water content; this allows the transition from solid to liquid at failure to be accomplished.

Normal engineering soil classifications are based entirely on size and this has obscured the contribution made to soil properties by particles of, or approaching, clay size but lacking clay mineral properties. The relationship of the various soil particles to each other is shown in Fig. 1 in which the bond-weight ratio R is plotted against particle size. The nature of R has been defined and discussed elsewhere⁷; it is the ratio of the cohesive force between particles to particle size. When $R=1$ the forces effectively balance; when $R<1$ the particle weight obscures the effect of the interparticle forces and the soil is termed cohesionless but when $R>1$ the system is cohesive and the particle interaction has more effect on the properties

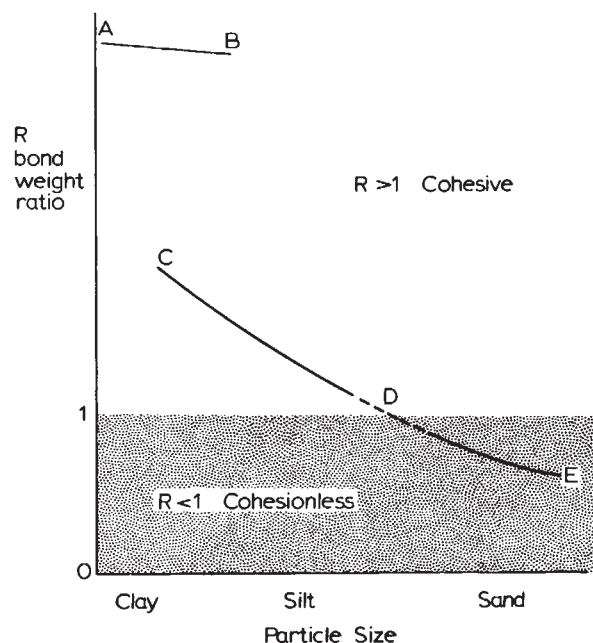


Fig. 1 Relationship of bond-weight ratio R to particle size for soil particles. AB , clay particles; CDE , non-clay particles (usually quartz in real soils).

than the weight of the particles. The line AB represents the clays with large R values; line CDE represents the non-clay mineral particles ranging from clay size to sand size. There is a certain lack of particles in the D region because of particle formation factors which result in the predominant quartz particles having a bimodal distribution⁸. Quickclays chiefly contain particles from around the C region and these, with short range bonds operating, determine the quickclay properties.

Three basic types of soil can be distinguished using criteria of particle size and interparticle bond type: (1) small particles, long range bonds (the real clays and clay soils); (2) small particles, short range bonds, and (3) larger particles, short range bonds (the sands). Previous investigators have tended to assume that quickclays were type 1 soils, with perhaps a slight tendency to "high silt content" but it is more satisfactory to consider them as type 2 soils. In mineralogical terms, however, quickclays are not really clays at all.

I. J. SMALLEY

Department of Civil Engineering,
University of Leeds, Leeds LS2 9JT

Received March 8; revised April 28, 1971.

- ¹ Penner, E., *Nature*, **197**, 347 (1963).
² Rosenqvist, I. Th., *Geotechnique*, **3**, 195 (1953).
³ Pusch, R., and Arnold, M., *Eng. Geol.*, **3**, 135 (1969).
⁴ Penner, E., *Canad. J. Earth Sci.*, **2**, 425 (1965).
⁵ Peck, R. B., Ireland, H. O., and Fry, T. S., *U. Illinois Civ. Eng. Dept. Soil Mech. Series*, No. 1 (1951).
⁶ Beland, J., *Proc. Geol. Assoc. Canada*, **8**, 143 (1956).
⁷ Smalley, I. J., *J. Soil Sci.*, **21**, 154 (1970).
⁸ Vita-Finzi, C., and Smalley, I. J., *J. Sediment. Petrol.*, **40**, 1367 (1970).

X-ray Diffraction Evidence for Crystalline Order and Isotropic Compression during the Shock-wave Process

WE have reported¹ observing X-rays diffracted from material which was undergoing shock-wave compression. The shock wave was generated by a conventional high-explosive, plane-wave generator. The four-channel scintillation detector used

in that experiment² demonstrated that diffraction had occurred, but it lacked angular resolution. To make this technique practical, a detector system capable of greater resolution had to be devised, and we have recently constructed a film cassette capable of withstanding the effects of the blast with no appreciable destruction of the film.

The experiment¹ has been repeated with film as a detector. We have now recorded, on film, X-ray diffraction from a material undergoing shock-wave compression.

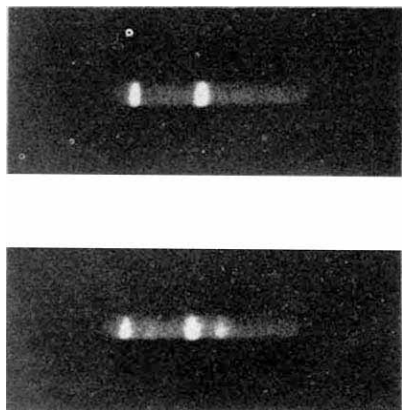


Fig. 1 X-ray diffraction records of the (111) and (200) reflexions of normal and shock-wave compressed LiF. Shock pressure is 180 kbar.

A photograph taken before detonation of the high explosive is presented as the top record in Fig. 1. Only the (111) and (200) reflexions of LiF are seen. The bottom record was taken such that the peak of the X-ray pulse from a pulsed X-ray source coincided with the arrival of the shock wave at the front surface of the sample. This shows, as well as reflexions typical of an uncompressed LiF unit cell with $a=4.027$ Å, a pair of weaker lines which can be attributed to the same reflexions of shock-compressed LiF with a unit cell dimension of $a=3.80$ Å. Christian³ previously obtained a pressure of 185 kb for a shock velocity of $6.61 \text{ mm } \mu\text{s}^{-1}$ and LiF sample density of 2.62 g cm^{-3} . Our sample density was 2.549 g cm^{-3} and we measured a shock velocity of $6.56 \text{ mm } \mu\text{s}^{-1}$; thus we would expect a lower pressure. The pressure derived from Christian's V/V_0 against pressure plot and our measured V/V_0 is 180 kb.

As stated in ref. 1, the observed angular shift demonstrates that the compression is isotropic on the unit cell basis and that equilibrium is reached very rapidly, as shown by the intensity of the lines from the shock-compressed state. Finally, this film record conclusively demonstrates that crystalline order is maintained behind the shock front at least for this particular shock pressure and sample.

Further details of this experiment and the film cassette will be published later. This work was performed under the auspices of the US Atomic Energy Commission.

QUINTIN JOHNSON
ARTHUR MITCHELL
L. EVANS

Lawrence Radiation Laboratory,
University of California,
Livermore, California 94550

Received April 14, 1971.

¹ Johnson, Q., Mitchell, A., Keeler, R. N., and Evans, L., *Phys. Rev. Lett.*, **25**, 1099 (1970).

² Johnson, Q., Mitchell, A., and Evans, L., *Rev. Sci. Instr.* (in the press, 1971).

³ Christian, R. H., Rep. 4900, Lawrence Radiation Laboratory, Livermore (1957).

Preparation of Ferrous Ferricyanide

We have synthesized ferrous ferricyanide, $\text{Fe}_3^{+2}(\text{Fe}^{\text{III}}(\text{CN})_6)_2$ by vacuum pyrolysis of Prussian blue (ferric ferrocyanide, $\text{Fe}_4^{+3}(\text{Fe}^{\text{II}}(\text{CN})_6)_3$)—see refs. 1 and 2. This completes the preparation of the curious quartet of iron-iron cyanides. The cation in each case is high spin ferrous (2+) or ferric (3+) iron, and the cyanide-surrounded anion is low spin ferrous (II) or ferric (III) iron. These compounds precipitate from solution when soluble salts are mixed; ferric chloride and potassium ferrocyanide, for example, yield Prussian blue (ferric ferrocyanide).

Attempts to synthesize ferrous ferricyanide always produced a material similar to Prussian blue, although the name Turnbull's blue was associated with the compound^{3,4}. An internal redox apparently occurs, and the electron moves from cation to anion. The effect of a mild vacuum pyrolysis is primarily that of forcing out the trapped water; small amounts of cyanogen and HCN are also released. The relief of this internal pressure presumably shrinks the lattice and reverses the redox process. (Externally applied pressure has also caused the appearance of ferrous iron in Prussian blue⁵.)

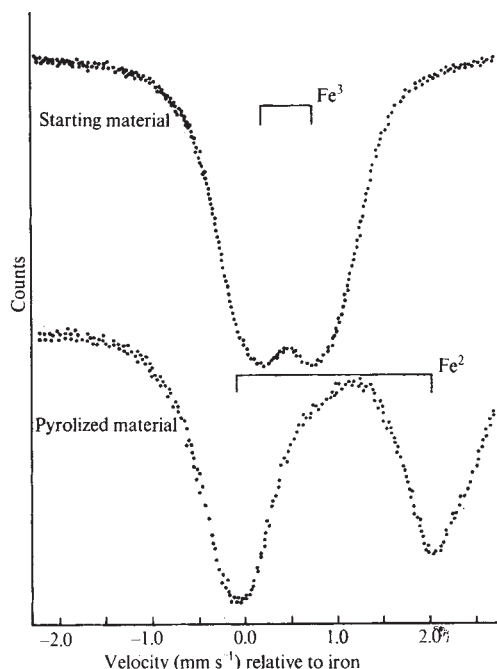
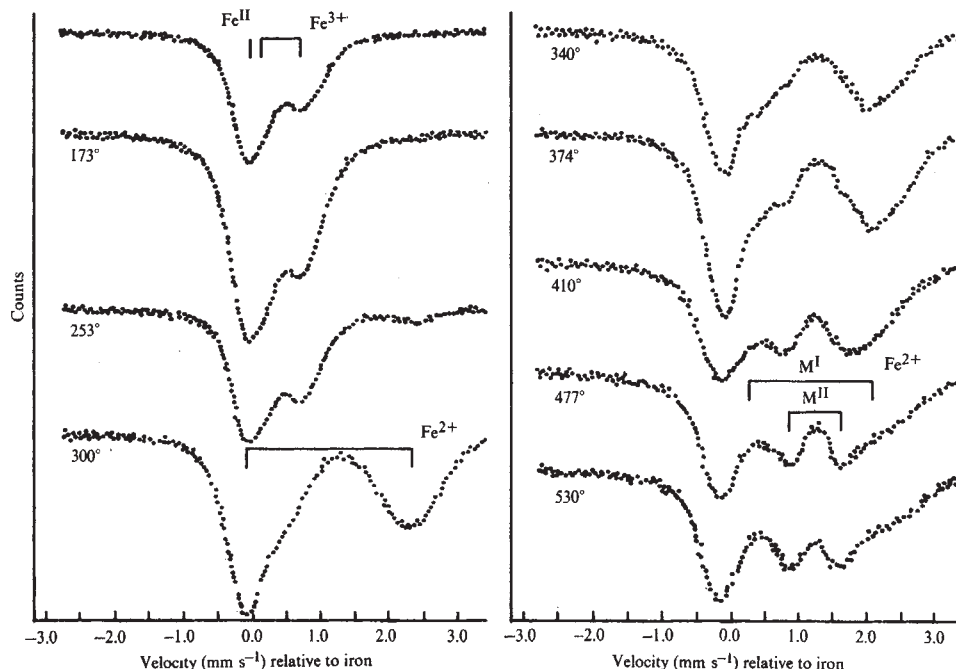


Fig. 1 Enriched Prussian blue: ferric enrichment pyrolysis at 400°C .

We have used ^{57}Fe Mössbauer spectroscopy for this analysis and the four kinds of iron yield distinctive patterns. The crystallites are also very small and tend to be amorphous to X-rays, but this does not obscure the Mössbauer information. In Fig. 1, the low spin iron is suppressed by ^{57}Fe enrichment of the high spin iron. The labelled ferric iron clearly changes on pyrolysis to ferrous iron. Similar results are obtained after enrichment of the anion iron.

The results for a series of pyrolyzed samples of Prussian blue are indicated in Fig. 2. The ferrous iron is present at two sites, metal I and metal II—metal I is present at the other end of each cyanide ligand. Metal II (half as much as metal I) is needed for charge neutrality, and is situated randomly in the large cages together with water molecules. As the water is driven out, the thermal motion of metal II increases and the quadrupole splitting diminishes. Mössbauer spectra at room temperature and above support this conclusion; the quadrupole splittings of metal I and metal II ferrous iron decrease with temperature. The metal II iron splitting decreases faster than the splitting of metal I.

Fig. 2 Mössbauer spectra of pyrolyzed Prussian blue at 77 K.



The ferrous ferricyanide is apparently quite stable at room temperature and is not affected by water. The ferric ferro-cyanide can be reconstituted by first treating with base and then with acid.

D. S. MURTY

Physics Department,
St Mary's University,
Halifax, Nova Scotia

J. G. COSGROVE
R. L. COLLINS

Physics Department,
The University of Texas at Austin,
Austin, Texas

Received February 25; revised April 27, 1971.

¹ Murty, D. S., and Collins, R. L., *Bull. Amer. Phys. Soc.*, Series II, **15**, 819 (1970).

² Collins, R. L., Murty, D. S., and Cosgrove, J. G., *Bull. Amer. Phys. Soc.*, Series II, **16**, 23 (1971).

³ Epstein, L. M., *J. Chem. Phys.*, **36**, 2731 (1962).

⁴ Maer, jun., K., Beasley, M. L., Collins, R. L., and Milligan, W. O., *J. Amer. Chem. Soc.*, **90**, 3201 (1968).

⁵ Fung, S. C., and Drickamer, H. G., *J. Chem. Phys.*, **51**, 4353 (1969).

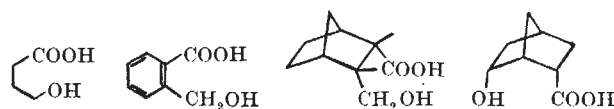
Orbital Steering and the Catalytic Power of Enzymes

A GREAT deal of interest and discussion has followed Koshland's suggestion that "orbital steering" is a major influence in the acceleration of chemical reactions by enzymes¹.

He has defined two effects influencing rates, the proximity and orientation effects², which are characteristic of enzyme catalysis and which might be responsible for the extreme specificity and catalytic power of enzymes. He has attempted to study the orientation effect in isolation and has postulated a very strong dependence of reaction rate on the precise relative orientation of atomic orbitals on the reacting atoms, calling this effect orbital steering¹. We have calculated the relevant overlap integrals for a model series of compounds in an attempt to discover whether the enormous rate differences observed in enzyme catalysis could reasonably be explained in this way.

Storm and Koshland¹ measured rates of intramolecular esterification for the series of compounds I-IV in Fig. 1, chosen to eliminate factors other than orientation. Rates were corrected for torsional strain in the rings formed and great variation in rate of lactonization was observed. The ratio of lactonization rate to thiolactonization rate also varied greatly, and since the only differences in these compounds is a small change in geometry of the active region and the substitution of oxygen by sulphur, the concept of orbital steering was used to explain this ratio of reaction rates, requiring the orbitals involved to have fine structure.

Using standard bond lengths and angles³ we have calculated the overlap integrals between the atoms involved in bond formation using the parameterized values of such methods as Segal's semi-empirical programme⁴⁻⁶. While the absolute values of these integrals are not correct, the compounds all contain the same active region, the only changes being in geometry and the replacement of oxygen by sulphur. We therefore



Corrected relative rates:

	I	II	III	IV
Lactonization	413	17	1,660	18,700
Ratio lactonization/thiolactonization	70	115	2.5×10^4	426
Internuclear separation C(OOH)-O(H)	1.675	2.378	1.979	2.251 Å
C(OOH)-S(H)	1.596	2.337	1.910	2.232 Å
Ratio of overlap integrals	ss	0.64	0.59	0.60
Oxygen/sulphur compounds	spσ	0.60	0.51	0.54
	ppσ	0.74	0.65	0.61
	ppπ	0.55	0.51	0.51

Fig. 1 A comparison of relative rates of esterification of model compounds with ratios of overlap integrals.

expect the ratio of overlap integrals for the sulphur compounds to those for oxygen compounds to be a reliable indication of the relative degrees of bonding overlap in the ring-closing species. If orbital steering is a significant influence on reaction rate we would expect this ratio of overlap integrals to be reflected in the ratio of lactonization rate to thiolactonization rate over the series of compounds I-IV, both these ratios are given in Fig. 1.

It is seen that the ratio of overlap integrals varies only slightly, in line with the physical chemist's picture of an orbital as rather diffuse and in sharp contrast with the fine structure mentioned by Storm and Koshland¹. It seems unlikely that the enormous differences in reaction rate ratio could be the result of such minor changes in differential orbital overlap.

These semi-empirical calculations do not seem to support the notion of orbital steering but more sophisticated calculations should be done before the idea is completely rejected.

G. N. J. PORT
W. G. RICHARDS

*Physical Chemistry Laboratory,
South Parks Road,
Oxford*

Received December 16, 1970.

¹ Storm, D. R., and Koshland, D. E., *Proc. US Nat. Acad. Sci.*, **66**, 445 (1970).

² Koshland, D. E., *J. Theoret. Biol.*, **2**, 75 (1962).

³ Sutton, L. E., *Interatomic Distances* (Special Publications of Chem. Soc., 11 (1958), 18 (1965)).

⁴ Pople, J. A., Santry, D. P., and Segal, G. A., *J. Chem. Phys.*, **43**, 5129 (1965).

⁵ Santry, D. P., and Segal, G. A., *J. Chem. Phys.*, **47**, 158 (1967).

⁶ Pople, J. A., and Segal, G. A., *J. Chem. Phys.*, **44**, 3289 (1966).

Solvophobic Interactions and Micelle Formation in Structure Forming Nonaqueous Solvents

HYDROPHOBIC interactions are important in maintaining the conformations of proteins and various macromolecules. The free energies of these interactions are entropic in origin, for the enthalpy changes are actually unfavourable at low temperatures ($\Delta H > 0$). It is generally believed that around the nonpolar parts of the solutes in contact with water there is an increased ordering¹⁻³ of the water molecules as a result of hydrogen bonding, and that on the formation of the hydrophobic bond this order is diminished so that there is a positive entropy change. We have recently shown⁴ that there are solvophobic interactions in ethylene glycol, a polar nonaqueous solvent, chiefly on the grounds of the formation of micelles by several cationic detergents and the limited solubility of hydrocarbons in this solvent.

We now report that there are solvophobic interactions in several other nonaqueous solvents such as glycerol, formamide, 2-aminoethanol, 1,3-propanediol, 1,4-butanediol, formic acid, ethylenediamine, 2-mercaptoethanol, 1,3-butanediol, 1,2-propanediol and 1-amino-2-propanol (class I). Such interactions seem not to exist in another eighteen solvents investigated, including N-methylformamide, dimethylformamide, diiodomethane (class II) and methanol, ethanol and toluene (class III).

The most important implication of these findings is that hydrophobic interaction is not a unique characteristic of water but a special case of a more general solvophobic interaction. Both must, however, be of similar origin. The hydrophobic interaction is supposed to be a consequence of the structure of water, and the evidence so far available suggests that structure effects may also be responsible for solvophobic interactions.

Our studies have shown that a nonionic detergent will aggregate into micelles only in the solvents belonging to class I.

The nonionic detergent used was *p,t*-nonylphenoxy polyethoxy ethanol, NPE₉ ('Igepal CO-630'), with an average of nine ethoxy residues in the polyethoxy chain. We measured the surface tensions of solutions of this detergent as a function of the concentration with a Rosano surface tensiometer which utilizes the Wilhelmy vertical plate technique—this does not involve the actual detachment of the plate at the time of measuring the surface tension. The method⁵ should therefore yield fairly accurate surface tension values. All measurements were carried out at 27.5° C and all the solvents were of reagent grades, except 1-amino-2-propanol which was of a practical variety.

The surface tensions of both class I and class II solvents were reduced by the dissolved detergent, but sharp breaks were observed in the graphs of surface tension against log detergent concentration only with class I solvents. We interpret these breaks in terms of the formation of micellar aggregates in these solvents, and have used them to determine the critical micelle concentrations (c.m.c.) of the detergent.

The surface tensions of class III solvents are actually increased by the dissolved detergent. No evidence of micelle formation in methanol and ethanol (class III) has, however, been obtained^{6,7}.

The surface tension of the liquid detergent alone is 37.2 dyne cm⁻¹. The surface tensions of all the class II solvents were higher than this, and those of all the class III solvents were lower. Thus the observed surface tension behaviour of the detergent in classes II and III solvents is that expected for mixtures of two liquids (the solvent and the liquid NPE₉).

The c.m.c. of NPE₉ in class I solvents were determined as described above and are summarized in Table 1. The free energies of micelle formation are calculated using the relationship

$$\Delta G_m = RT \ln (\text{c.m.c.}) \quad (1)$$

even though the size of the micelles is unknown at present. Also the very high c.m.c. values in some of the solvents may invalidate the use of equation 1 because the monomer activity would be quite different from the monomer concentration at the c.m.c. Such ΔG values should therefore be taken as only approximate. The results for water and ethylene glycol are also included in Table 1 for comparison.

Solvophobic interactions seem to be most pronounced in water, and to a slightly smaller extent in glycerol. Both water and glycerol have very high concentrations of OH groups per unit volume, so it can be argued that the solvophobic interaction is caused by an incompatibility of the OH groups of the solvent molecules with the hydrocarbon parts of the nonpolar solutes. Comparisons between ethylene glycol and 2-aminoethanol, and between formic acid and formamide, indicate, however, that there is no straightforward relationship between solvophobic interaction and the OH group density of the solvent molecules. But a comparison of ethylenediamine with ethylene glycol and 2-aminoethanol shows that the replacement of both the OH groups by NH₂ groups does greatly reduce the solvophobic effect.

The idea that the solvophobicity of the hydrocarbon parts of the detergent molecules is the driving force for formation of micelles is given support by the fact that the solubilities of hydrocarbons⁸ in class I solvents are either poor or limited, whereas they are miscible in all proportions with the solvents of classes II and III.

The structures of these micelles are very likely to resemble those of micelles in water, which have an oily interior and the polar groups exposed to the solvent, rather than those of the "inverted" micelles^{9,10} which are known to form in hydrocarbon solvents such as toluene, n-decane and so on because large and favourable interactions may be expected between the polar heads of the detergents and the polar solvent molecules. This would be particularly true for cationic detergents (ref. 4 and my unpublished data) which have been found to form micelles in ethylene glycol⁴, glycerol, formamide and 1,2-propanediol.

Table 1 Observed Solvophobic Effects and Bulk Properties of the Solvents

Solvent	Dielectric constant	Surface tension (dynes cm ⁻¹)	δ (calorie ^{1/2} cm ^{-3/2})	Critical micelle concentration of NPE ₉ at 27.5° C (mol fraction)	$-\Delta G_m$ (kcalorie mol ⁻¹)
Class I					
Water	78.5	72	23.4	5.6×10^{-5}	5.84
Glycerol	42.5	63	15.4	8.7×10^{-5}	5.43
Ethylene glycol	37.7	48.9	15.5	1.25×10^{-2}	2.61
2-Aminoethanol	—	50.3	—	1.53×10^{-2}	2.49
Formamide	109.5	57.6	19.4	1.57×10^{-2}	2.48
1,3-Propanediol	—	49.2	13.5	6.3×10^{-2}	1.65
1,4-Butanediol	—	47.4	—	1.60×10^{-1}	1.09
Ethylenediamine	14.2	42.9	12.0	3.39×10^{-1}	0.65
1,3-Butanediol	—	39.1	—	4.0×10^{-1}	0.54
2-Mercaptoethanol	—	45.7	—	4.0×10^{-1}	0.54
Formic acid	58.5	38.1	—	4.5×10^{-1}	0.48
1,2-Propanediol	—	38.0	13.3	5.0×10^{-1}	0.41
1-Amino-2-propanol	—	36.5	—	5.0×10^{-1}	0.41
Class II					
N-Methylformamide	182.4	40	16.1	—	—
Dimethylformamide	36.7	37.7	12.2	—	—
Diiodomethane	—	50.7	11.8	—	—
Class III					
Methanol	32.6	22.6	17.2	—	—
Ethanol	24.3	22.3	12.9	—	—
Toluene	2.4	28.5	8.9	—	—

Attempts were made to correlate the observed solvophobic effects with the bulk properties of the solvents, such as dielectric constants, solvent surface tensions, and solubility parameters^{8,11}. The lack of any such correlation is evident from Table 1.

One noticeable feature of all the micelle forming solvents is that the molecules of these solvents have two or more potential hydrogen bonding centres, and are therefore most likely to be capable of forming three-dimensional hydrogen bonded network structures. We therefore postulate that these molecules would be more structured or ordered around the hydrocarbon parts of the detergent molecules. After removal of this hydrocarbon moiety from the solvent environment to the oily interior of the micelles, this order would be diminished and there would be a positive entropy change.

Several arguments can be made in support of the mechanism postulated above.

(a) The enthalpy of micelle formation for NPE₉ in ethylene glycol (my unpublished data), as estimated from the temperature dependence of the c.m.c., is close to zero in the range 25°–45° C. This shows that the free energy of micelle formation in this system (Table 1) is primarily entropic in origin.

A more direct way of proving the entropic origin of the free energy of solvophobic interactions would be to determine the solubilities of hydrocarbons as a function of temperature in these solvents. This is now being carried out in this laboratory.

(b) Formamide—a micelle forming solvent—is already known to contain some structure in the liquid state¹² that resembles the structure of solid formamide¹³.

(c) N-Methylformamide¹⁴, methanol¹⁵ and ethanol¹⁵ are known to contain only linear hydrogen bonded aggregates, and micelles do not form in any of these solvents.

(d) There is a drastic reduction in the solvophobic effects whenever a pendant type of methyl group is present in the solvent molecules. The comparisons of 1,3-propanediol with 1,2-propanediol, 1,4-butanediol with 1,3-butanediol and 2-aminoethanol with 1-amino-2-propanol are most interesting in this respect. The differences between these solvent pairs can be explained in terms of reductions in structure formation in 1,2-propanediol, 1,3-butanediol and 1-amino-2-propanol caused by the steric hindrances due to the CH₃ groups.

(e) The urea molecule also has three potential hydrogen bonding centres and could therefore be placed in the same

category as the class I solvents. It has been suggested that in aqueous solutions, urea can participate in extensive hydrogen bonding¹⁶ with water, and that mixed urea–water clusters¹⁷ may be formed. It is therefore not surprising that micelles continue to form in 8 M¹⁶ and even 10 M (my data, submitted for publication) aqueous urea solutions.

(f) After this work was completed, it was learnt that there have been some independent studies of the micelle formation and the solubility of a hydrocarbon in formamide¹⁸. These results also confirm the entropic origin of the solvophobic interaction in formamide.

Much work needs to be done before the nature of solvophobic interactions can be understood with certainty. These investigations are also undoubtedly important for the better understanding of hydrophobic interactions—if, for example, future research shows that solvent structure effects are not involved in solvophobic interactions, it might be necessary to look for a different interpretation for hydrophobic interactions as well. A much better understanding of the interactions of these solvents with proteins and other macromolecules would also result.

Studies of the structures of the micelle forming solvents and of the various aspects of the interactions of these solvents with hydrocarbons, detergents, proteins and other macromolecules have also been undertaken in this laboratory.

This work was supported by the US National Science Foundation. I thank Professor H. S. Frank for discussions and Professor G. Némethy for discussion and interest in this work.

ASHOKA RAY

The Rockefeller University,
New York, NY 10021

Received December 4, 1970; revised April 6, 1971.

¹ Frank, H. S., and Evans, M. W., *J. Chem. Phys.*, **13**, 507 (1945).

² Kauzmann, W., *Adv. Protein Chem.*, **14**, 1 (1959).

³ Némethy, G., and Scheraga, H., *J. Chem. Phys.*, **36**, 3401 (1962).

⁴ Ray, A., *J. Amer. Chem. Soc.*, **91**, 6511 (1969).

⁵ Adam, N. K., *The Physics and Chemistry of Surfaces*, 383 (Dover, New York, 1968).

⁶ Kitahara, A., Kobayashi, T., and Tachibana, T., *J. Phys. Chem.*, **66**, 363 (1962).

- ⁷ Little, R. C., and Singleterry, C. R., *J. Phys. Chem.*, **68**, 2709 (1964).
- ⁸ *The Handbook of Chemistry and Physics*, fiftieth ed. (The Chemical Rubber Co., 1969).
- ⁹ Heilweil, I. J., *J. Colloid Sci.*, **19**, 105 (1964).
- ¹⁰ Fryar, A. J., and Kaufman, S., *J. Colloid and Interface Sci.*, **29**, 444 (1969).
- ¹¹ Hildebrand, J. H., and Scott, R. L., *The Solubility of Non-electrolytes*, third ed. (Dover, New York, 1964).
- ¹² DeSando, R. J., and Brown, G. H., *J. Phys. Chem.*, **72**, 1088 (1968).
- ¹³ Ladell, J., and Post, B., *Acta Cryst.*, **7**, 559 (1954).
- ¹⁴ Leader, G. R., and Gormley, J. F., *J. Amer. Chem. Soc.*, **73**, 5731 (1951).
- ¹⁵ Franks, F., and Ives, D. J. G., *Quart. Rev.*, **20**, 1 (1966).
- ¹⁶ Mukerjee, P., and Ray, A., *J. Phys. Chem.*, **67**, 190 (1963).
- ¹⁷ Abu-Hamdiyyah, M., *J. Phys. Chem.*, **69**, 2720 (1965).
- ¹⁸ McDonald, C., *J. Pharm. Pharmacol.*, **22**, 10, 774 (1970).

BIOLOGICAL SCIENCES

Periodicity of Speech in Parkinsonism

IMPAIRMENT of speech, leading sometimes to unintelligibility, was recognized by Parkinson¹ to be part of the disease which now bears his name. Reduced voice volume, dysarthria and prosodic monotony are common manifestations of Parkinsonism² and are generally attributed to hypokinesia, a cardinal feature of the disease, the physiological basis of which is ill understood. The term itself is difficult of definition, but it is generally thought to imply a delay in initiation of movement combined with slowness and restriction in amplitude of movements once initiated.

Quantitative assessment of hypokinesia is difficult but an objective analysis of speech function offers a basis for investigation. Joshua Steele³ emphasized the importance of pauses in the rhythm of speech. Little study has been made of the relationship of sounds to pauses in the speech of Parkinsonian patients. L-Dopa in large oral doses has been shown by many investigators to ameliorate Parkinsonism and particularly to improve the hypokinetic defects thereof. We have studied the periodicity of phonation and pauses in patients suffering from Parkinsonism and the alterations produced in these parameters by treatment with L-dopa.

Twenty Parkinsonian patients, aged between 41 and 76, were investigated. Sixteen suffered from the idiopathic disease, paralysis agitans, and two of these had earlier been treated by bilateral stereotactic thalamotomies. The remaining four patients suffered from the post-encephalitic form of Parkinsonism. All derived perceptible benefit from treatment with L-dopa as gauged by their general functional capacity and mobility⁴. This was the only criterion for inclusion. Patients were not selected because of the nature of their speech defect, nor because of a specifically noteworthy improvement in speech function. They were all observed in hospital during the period of treatment. Recordings of their speech were made before treatment was instituted, and again when clinically they were adjudged to have improved, usually after taking L-dopa for 3 or 4 weeks.

Extensive studies of spontaneous speech and reading were made at each recording session after an introductory conversation designed to minimize the inhibitions attendant on the test situation. During each session the patient was asked to count from one to ten, with no instructions about loudness or the rate of utterance required. We thought that counting to ten would be the most apposite test for a study of speech periodicity. It is a universally familiar activity with constituents which are, with one exception, monosyllabic. In the act of counting there are no constraints on the rhythms of speech determined by punctuation delays. Counting conforms to Hughlings Jackson's⁵ "automatic" utterance. It is, therefore, virtually free of those hesitations consequent on the formulation of "superior" or

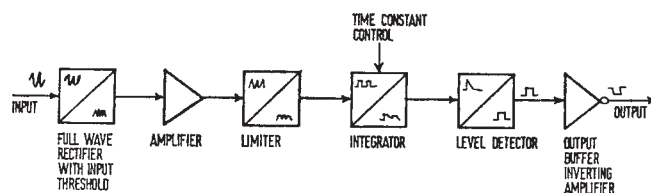


Fig. 1 Diagram of signal detector.

propositional speech which have been extensively studied by Goldman Eisler⁶.

The recordings were played through a signal detector which was triggered by sound (Fig. 1). For accurate measurements of phonations this apparatus required that the patient's speech should be at least 20 dB louder than the background noise level. This provided a signal of 10 V, the input threshold of the detector being 1 V. In practice these conditions were achieved.

The initial delay to production of the first digit was not assessed. The phonation times for each of the ten digits and the duration of each of the nine intervening pauses were measured. When, as occasionally occurred, two phonations ran together without detectable pause, the total phonation time was halved and this value was allotted to each digit. Means and standard deviations for the duration of each phonation and each pause were calculated for each patient before treatment was started and again while on L-dopa, and the results are shown in Table 1.

These data show that the mean phonation time for single digits decreased significantly ($P < 0.01$) after treatment (339 ± 85 ms) compared with the pretreatment figure (389 ± 107 ms). In addition the variability of the phonations was reduced. Before treatment the standard deviations of individual patients give a mean value for the group of 139 ± 55 ms. After treatment the corresponding figure is 108 ± 36 ms. The change is significant at the 1% level. After treatment the mean duration of each pause (440 ± 227 ms) was longer than the mean in untreated patients (372 ± 194). This difference, however, is significant at only the 10% level of confidence.

Six patients, 2, 5, 13 and 20, who had suffered from the post-encephalitic form of Parkinsonism and 8 and 19 who had earlier undergone bilateral thalamotomies, were assessed clinically as showing little or no improvement in their speech after treatment with L-dopa. If they are subtracted from the

Table 1 Duration of Phonation of Parkinsonian Patients before and after Treatment with L-dopa

Patient No.	Duration of phonations (ms)		Duration of pauses (ms)	
	Before treatment Mean s.d.	After treatment Mean s.d.	Before treatment Mean s.d.	After treatment Mean s.d.
1	689 ± 199	522 ± 125	382 ± 149	638 ± 80
2	398 ± 153	260 ± 126	569 ± 157	480 ± 142
3	428 ± 104	408 ± 95	430 ± 114	411 ± 108
4	245 ± 142	230 ± 103	138 ± 74	394 ± 68
5	415 ± 142	405 ± 95	172 ± 100	152 ± 78
6	314 ± 106	404 ± 126	640 ± 144	827 ± 192
7	305 ± 87	322 ± 70	650 ± 142	750 ± 150
8	302 ± 116	242 ± 56	470 ± 195	203 ± 50
9	366 ± 115	283 ± 101	520 ± 90	688 ± 70
10	320 ± 112	261 ± 132	705 ± 133	493 ± 113
11	464 ± 82	255 ± 61	328 ± 181	504 ± 114
12	315 ± 123	285 ± 98	617 ± 332	722 ± 184
13	285 ± 128	286 ± 106	302 ± 138	155 ± 45
14	385 ± 110	330 ± 120	265 ± 128	330 ± 149
15	368 ± 199	350 ± 110	216 ± 72	205 ± 93
16	588 ± 182	473 ± 71	355 ± 138	708 ± 118
17	366 ± 168	336 ± 93	101 ± 36	365 ± 154
18	496 ± 85	483 ± 118	158 ± 75	211 ± 100
19	415 ± 321	350 ± 222	132 ± 97	86 ± 33
20	320 ± 106	302 ± 139	288 ± 72	475 ± 84
Group	389 ± 107	339 ± 85	372 ± 194	440 ± 227

group then the lengthening of the duration of pauses in the remainder becomes significant at the 1% level. There is too an apparent narrowing of the variation of the pause durations. Before treatment the mean of the standard deviations for the whole group of twenty patients is 128 ± 62 ms; after treatment 106 ± 44 ms. However, this apparent change does not achieve significance at the 10% level of confidence.

Estimates of the rates of speech during counting can be derived from these results. In patients before treatment the mean rate was eighty-nine words per minute, and after treatment eighty-eight words per minute. These figures are surprisingly similar to the average rate of eighty-seven words per minute which we found in twenty subjects, matched for age, who suffered from no neurological lesion and who were asked to count from one to ten in similar conditions to the Parkinsonian patients.

The speech of most of the patients in this group became more intelligible after treatment with L-dopa. Improvement in voice volume and in dysarthria contributed to this, but the changes in periodicity shown above are also important. The rate of speech does not alter, but each digit takes a significantly shorter time for its utterance and is separated from its preceding and following numbers by lengthened pauses. There is too a tendency for both phonations and pauses to be more regularly distributed after treatment. Shorter, crisper sounds separated by better defined pauses would be expected to improve the clarity of speech, and so they do. These results are compatible with the observations of others⁷ about the improvement of speech function in Parkinsonian patients treated with L-dopa. They indicate that this improvement is particularly manifest in patients with paralysis agitans. There is a suggestion, which needs further study, that in those patients with the post-encephalitic disease and in those who have undergone stereotactic thalamotomy, L-dopa may be less effective in improving speech.

The concept of motor deficit in Parkinsonism as a simple slowing and paucity of movements is inadequate to explain these observed changes in speech periodicity. The more rapid phonations are explicable on the basis of rectification of hypokinesia; but the greater separation of phonations which our results suggest implies a process more complex than a mere speeding up of motor activity. Nor is this consideration peculiar to speech function. The gait of many Parkinsonians is rapid even though the individual steps be short. Indeed, some patients show a tendency to involuntary acceleration, or festination, of their gait. In such patients L-dopa often produces not a more rapid progress but a more controlled gait, longer steps being taken more slowly. As Denny-Brown⁸ has pointed out, there is in Parkinsonism a defect in the general quality of motor performance.

It is interesting to speculate, in the light of recent biochemical and physiological findings on the underlying mechanisms of the speech disturbances and their amelioration by L-dopa. There is overwhelming evidence that a decrease in striatal dopamine is an almost invariable factor in the production of the Parkinsonian syndrome^{9,10}. Dopamine applied microelectrophoretically leads to inhibition in many neurones in the caudate nucleus¹¹.

Mettler¹² has shown that bilateral lesions of the caudate nuclei in apes produce over-activity, or hyperkinesia, which is driven by proprioception impulses. The principal outflow from the striatum is to the pallidum which itself lies in the efferent pathway from the thalamus for movement evoked by proprioceptive impulses. Purpura *et al.*¹³ have demonstrated that stimulation of the caudate inhibits the outflow of impulses from the pallidum. Mettler¹⁴ concludes that the striatum normally inhibits the thalamopallidal circuits and hence allows the organism to make choices and not to be dominated by sensory, and particularly proprioceptive, stimuli.

Lenneberg¹⁵ postulates that if delays are introduced into a feedback monitoring system for speech the cues for the beginning of the next cycle of activity are delayed and hence

there is a prolongation of the ongoing motor activity. This leads to a drawing out of vowels.

We suggest that the principal lesion in Parkinsonism lies in the striatum, the malfunction of which releases the thalamopallidal circuit from inhibition and hence kinaesthetic information from the speech organs is submerged in random proprioceptive impulses. The choice of the next pattern of motor activation is delayed because of this confusion of information which effectively leads to the prolongation of the ongoing phonation. Restoration of the inhibitory striatal action, by activation of dopaminergic neurones, restores a more rapid selection of the cues for the next stage of motor activation, thus shortening the phonation times for individual words and lengthening the pauses. This hypothesis ignores the effect of auditory feedback which would, of course, be normal in most Parkinsonian patients. It seems likely, however, that kinaesthetic impulses from the muscles of the speech organs function as a monitoring mechanism, additional to auditory feedback, for spoken speech. Such a theory fits the facts of our observed data.

We thank Professor R. C. Oldfield for advice and encouragement, Mr Alistair Kinloch for technical assistance, and Dr H. Holgate of Roche Products Limited for L-dopa.

C. MAWDSLEY

Department of Medical Neurology,
University of Edinburgh

C. V. GAMSU

MRC Speech and Communication Unit,
University of Edinburgh

Received February 1, 1971.

- ¹ Parkinson, J., *An Essay on the Shaking Palsy* (Sherwood, Meely and Jones, London, 1817).
- ² Canter, G. J., thesis, Univ. Michigan (1961).
- ³ Steele, J., *Prosodia Rationalis* (Nichols, London, 1779).
- ⁴ Mawdsley, C., *Brit. Med. J.*, **1**, 331 (1970).
- ⁵ Jackson, H. J., *Brain*, **1**, 304 (1878).
- ⁶ Goldman Eisler, F., *Psycholinguistics* (Academic Press, London and New York, 1968).
- ⁷ Rigrodsky, S., and Morrison, E. B., *J. Amer. Ger. Soc.*, **18**, 142 (1970).
- ⁸ Denny-Brown, D. E., in *Diseases of the Basal Ganglia, Handbook of Clinical Neurology*, **6** (edit. by Vinken, P. J., and Bruyn, G. W.) (North-Holland, Amsterdam, 1968).
- ⁹ Ehringer, H., and Hornykiewicz, O., *Klin. Wschr.*, **38**, 1236 (1960).
- ¹⁰ Bernheimer, H., Birkmayer, W., and Hornykiewicz, O., *Klin. Wschr.*, **39**, 1056 (1961).
- ¹¹ Bloom, F. E., Costa, E., and Salmoiraghi, G. C., *J. Pharmacol. Exp. Therap.*, **150**, 244 (1965).
- ¹² Mettler, F. A., *J. Comp. Neurol.*, **83**, 93 (1945).
- ¹³ Purpura, D. P., Frigyesi, T. L., and Malliani, A., in *Neurophysiological Basis of Normal and Abnormal Motor Activities* (edit. by Yahr, M. D., and Purpura, D. P.) (Raven Press, New York, 1967).
- ¹⁴ Mettler, F. A., in *Neurophysiological Basis of Normal and Abnormal Motor Activities* (edit. by Yahr, M. D., and Purpura, D. P.) (Raven Press, New York, 1967).
- ¹⁵ Lenneberg, E. H., *Biological Foundations of Language* (Wiley, New York, 1967).

Effect of Radiation on the Germination of Spores of *Dictyostelium discoideum*

SPORES of the cellular slime mould *Dictyostelium discoideum* will germinate in liquid culture at relatively high densities ($< 5 \times 10^6$ spores/ml.), especially if they are provided with bacteria on which the amoeboid cells can feed¹. When the spore density becomes very high ($> 2 \times 10^7$ spores/ml.), however, germination will not take place at all. The cause is thought to be an inhibitor secreted by the spores themselves².

Cellular slime moulds are monocellular amoeboid organisms that multiply by fission. When the food supply is exhausted,

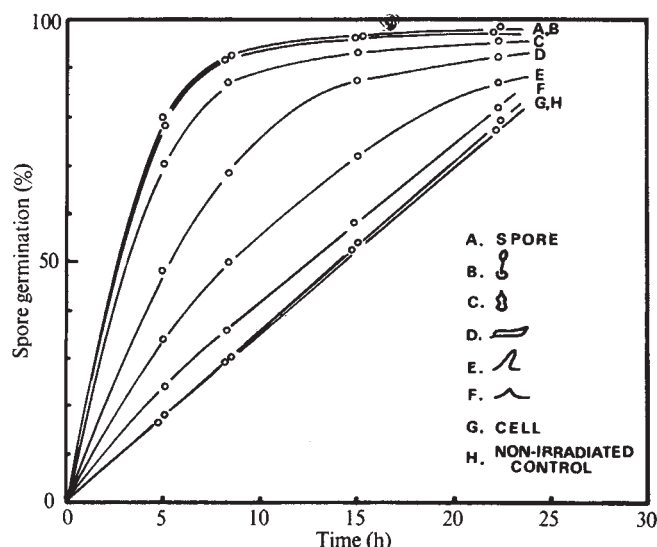


Fig. 1 Germination rate of spores which matured after irradiation (^{60}Co γ -ray, 7.0×10^4 r.) at the following morphological stages: A, matured spores; B, fruiting bodies; C, culmination stage; D, pseudoplasmodium; E, finger-like aggregation; F, aggregation; G, starvation stage; H, non-irradiated spores.

the amoeboid cells gather to form aggregates and then fruiting bodies which bear spores^{5,6}. Dearing³ and I⁴ have found that when a suitable dose of γ -rays is administered to spores, they will germinate at high density. I now report that γ -rays are effective in increasing germination at high densities even when administered before spore formation.

Spores of strain NC-4 of *D. discoideum* were cultured with *Aerobacter aerogenes* on nutrient agar; development was synchronized by the 'Millipore' filter method⁷. The multicellular aggregates in different stages of development were irradiated with ^{60}Co γ -rays (7.0×10^4 r./h for 1 h), and incubated to form fruiting bodies. After 4 days of fruiting body formation, spores were collected and shake cultured at 22°C at a density of 3×10^6 spores/ml. with bacteria. Small amounts of the samples were taken at definite time intervals and the germination rate was determined⁴.

Fig. 1 shows the results obtained. Curve H is the germination rate of the non-irradiated spores; approximately 20 h were required for 80% of the spores to germinate. When the spores were initially irradiated with γ -rays, the germination rate increased remarkably (curves A and B); the time required for 80% germination was decreased to approximately 5 h. Irradiation at earlier stages also increased the germination rate, but the effect was not as large (curves C, D and E). A very small increase in germination rate was observed when the cells in the aggregation phase were irradiated (curve F). Irradiation of the cells before the aggregation phase, however, did not give any increase (curve G). Fig. 1 shows that the germination rate increased more markedly when the irradiation was given at later stages of development.

The mechanism by which γ -rays increase the germination rate of spores is not yet clear. From the experiments described here it seems probable that the observed increase does not result from the breakdown of the germination inhibitor by γ -rays, but should be attributed to the deterioration of some function such as the secreting or receiving power of the spore. It is not at all clear that just one mechanism is involved. To determine the mechanism or mechanisms involved, further experiments, such as the determination of inhibitor content and the permeability of the inhibitor through the spore wall for irradiated and non-irradiated cells, are being planned.

Y. HASHIMOTO

Genetics Laboratory,
Isotope Research Institute,
Fukazawa, Setagaya, Tokyo 158

Received December 21, 1970.

- ¹ Cotter, D. A., and Raper, K. B., *Proc. US Nat. Acad. Sci.*, **56**, 880 (1966).
- ² Cotter, D. A., and Raper, K. B., *J. Bact.*, **96**, 1680 (1968).
- ³ Dearing, R. A., *J. Bact.* (in the press).
- ⁴ Hashimoto, Y., and Yanagisawa, K., *Radiat. Res.* (in the press).
- ⁵ Raper, K. B., *J. Agric. Res.*, **58**, 157 (1939).
- ⁶ Bonner, J. T., *The Cellular Slime Molds*, second ed. (Princeton University, Princeton, 1967).
- ⁷ Sussman, M., *Method. Cell Physiol.*, **11**, 397 (1966).

Structural Changes in Myosin induced by Vitamin E Dystrophy

THE modification of the soluble enzyme composition of muscle that takes place during development seems to be an adaptive response to changes in the functional activity of the muscle¹. Similar adaptive response occurs in the myofibrillar system for the enzymatic and structural properties of myosin are characteristic of the activity pattern²⁻⁴ of the muscle from which it is derived. An apparent reversal of the normal development process occurs during some types of muscular dystrophy⁵ in that certain isoenzymes are found in the proportions typical of foetal tissue. The effect of this reversion on the enzymic and structural properties of myosin in dystrophy induced by vitamin E deficiency has been studied.

Changes in primary structure were observed in the myosin isolated from the mixed skeletal muscle of both New Zealand White and Dutch strains of rabbit kept for 6-16 weeks on a diet deficient in α -tocopherol^{6,7}. In comparison with appropriate controls, the myosin isolated from dystrophic muscle contained smaller amounts of 3-methyl histidine and they continued to decrease during the period of vitamin E deficiency (Table 1). The myosin was carefully purified by our standard procedure² with 15 mM 2-mercaptoethanol present throughout, followed by chromatography on DEAE-Sephadex A-50⁸ in the presence of 1 mM dithiothreitol. No contaminants present in sufficiently large amounts to affect the analytical data could be detected on polyacrylamide gel electrophoresis in the presence of 8 M urea.

The results suggest that the dystrophic muscle synthesizes more of the foetal type of myosin which contains no 3-methyl histidine⁹. Limited studies indicated that the N^ε-mono- and N^ε-trimethyl lysine content of skeletal muscle myosin¹⁰ did not change during the period of vitamin E deprivation studied.

When the low molecular weight subunits of myosin were examined by gel electrophoresis in 8 M urea we found that the relative amount of the fastest component, MI_1 ⁴, was less (as judged by the intensity of amido schwarz stain) than that present in the normal preparation (Fig. 1). By the same criterion, no obvious differences in the relative amounts of the

Table 1 3-Methyl Histidine Content of Myosin Isolated from Dystrophic Muscle

	Time on diet (weeks)	3 MeHis/mol myosin
Standard diet		1.64
Deficient diet + vitamin E	10	1.53
Deficient diet	7	0.75
	8	1.01
	9	0.73
	10	0.66
	13	0.69
	16	0.55

Muscle was isolated as described in text from mixed skeletal muscle of New Zealand White rabbits. 10 mg of α -tocopherol was administered orally on alternate days to control animals. Analyses were carried out as described before⁹.

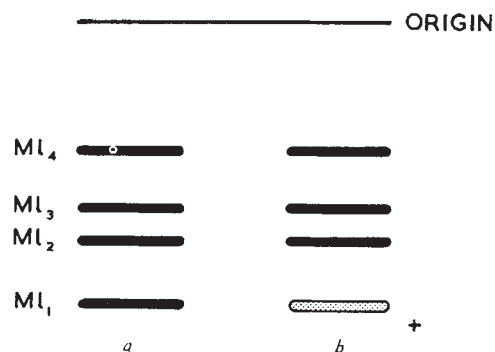


Fig. 1 Diagrammatic representation of the band patterns obtained from polyacrylamide gel electrophoresis of the low molecular weight components of myosin isolated from normal and vitamin E dystrophic rabbit skeletal muscle. *a*, Normal; *b*, vitamin E dystrophic. Electrophoresis was carried out on 10% acrylamide gels containing 8 M urea as described in ref. 4.

other three low molecular weight components could be detected. Subfragment I¹¹ prepared from dystrophic myosin by papain digestion¹² differed from the normal preparation in behaviour on gel electrophoresis in conditions in which it did not dissociate into the light and heavy chains, that is, on an 8% acrylamide gel in 40% glycerol containing 20 mM Tris-glycine buffer, pH 8.6. Whereas the normal preparation migrated as two poorly resolved bands, both of which stained for ATPase activity¹³, the subfragment I preparations obtained from the vitamin E dystrophic muscle showed only one protein band; this possessed ATPase activity. The single band present in subfragment I from dystrophic muscle corresponded to the faster of the two bands seen in normal preparations. Changes in the low molecular weight components of subfragment I paralleled those observed in myosin itself.

The above structural modifications were accompanied by changes in biological activity: preliminary results indicated that both the activity of the specific Ca²⁺-stimulated ATPase and of subfragment I in the myosin isolated from dystrophic muscle were significantly lower than those of the corresponding preparations from normal muscle (Table 2). The reduction in enzyme activity was presumed not to have been caused by oxidation of thiol groups in the dystrophic myosin¹⁴ because the preparations were isolated and purified in 15 mM 2-mercaptoethanol.

Table 2 Specific Ca²⁺ Stimulated ATPase Activities of Myosin isolated from Dystrophic Muscle

	Normal muscle	Dystrophic muscle
Myosin	26.7 27.5	9.75 8.5
Subfragment I	110 96.0	48.0 51.2

Assays were carried out in 25 mM Tris-HCl (pH 7.6), 2.5 mM Tris-ATP, 5 mM CaCl₂, 2.5 mM ATP and 0.5 M KCl. ATPase activities are expressed in $\mu\text{g P}_i/\text{mg protein/min}$ and represent the results obtained with two different preparations of dystrophic and control myosin.

The 3-methyl histidine content, ATPase activity and band pattern of the low molecular weight components of myosin isolated from white skeletal muscle change as the vitamin E dystrophy progresses, becoming similar to those of myosin isolated from red muscle of the adult and skeletal muscle of the neonatal rabbit. These changes are considered to represent an increase in the proportion of the foetal type of myosin associated with the progression of the dystrophic condition. Low 3-methyl histidine content, reduced amount of the ML₁ subunit and lower ATPase activity have all been described as characteristic of this form of myosin⁵. The reduction in 3-

methyl histidine content implies a change in primary structure of the heavy chains, for this residue is absent from the light chains of myosin¹⁵. The light chain ML₄ is probably not identical to the light chain fraction that can be removed from myosin by treatment with 5,5'-dithiobis-(2-nitrobenzoic acid)^{16,17} without loss of enzyme activity⁴.

The change in myosin composition to that more characteristic of the foetal tissue is similar to modifications that occur in the isoenzymes of creatine kinase and lactate dehydrogenase in dystrophic conditions of genetic origin¹⁸. Changes in the proportions of the different types of myosin built into the structure of the A filaments, however, introduce additional complications in the biosynthetic pathway compared with those involved in the change in the isoenzyme pattern of the soluble sarcoplasmic enzymes. From this point of view it is interesting that no significant change of the 3-methyl histidine content of actin could be detected during vitamin E dystrophy. These findings imply that the structure of myosin changes as the dystrophy progresses whereas that of actin does not.

This investigation was supported in part by grants from the Muscular Dystrophy Associations of America Inc. and the Medical Research Council.

G. E. LOBLEY
S. V. PERRY
D. STONE

Department of Biochemistry,
University of Birmingham

Received December 18, 1970; revised February 22, 1971.

- Kendrick-Jones, J., and Perry, S. V., *Biochem. J.*, **103**, 207 (1967).
- Trayer, I. P., and Perry, S. V., *Biochem. J.*, **345**, 87 (1966).
- Barany, M., *The Contractile Process*, supplement to *J. Gen. Physiol.*, **50**, 197 (1967).
- Perrie, W. T., and Perry, S. V., *Biochem. J.*, **119**, 31 (1970).
- Perry, S. V., *J. Neurol. Sci.* (in the press).
- Green, J., Diplock, A. T., Bunyan, J., McHale, D., and Muthy, I. R., *Brit. J. Nutr.*, **21**, 69 (1967).
- Bunyan, J., Green, J., and Diplock, A. T., *Brit. J. Nutr.*, **21**, 137 (1967).
- Richards, E. G., Chung, C. S., Menzel, D. B., and Olcott, H. S., *Biochemistry*, **6**, 528 (1967).
- Trayer, I. P., Harris, C. I., and Perry, S. V., *Nature*, **217**, 452 (1968).
- Hardy, M. F., Harris, C. I., Perry, S. V., and Stone, D., *Biochem. J.*, **120**, 653 (1970).
- Mueller, H., and Perry, S. V., *Biochem. J.*, **85**, 431 (1962).
- Lowey, S., Slayter, H. S., Weeds, A. G., and Baker, H., *J. Mol. Biol.*, **42**, 1 (1969).
- Perry, S. V., Hardy, M. F., and Stone, D., *Proc. Eighth Intern. Cong. Biochem.*, **35** (1970).
- Stracher, A., and Dow, J., *Proc. Eighth Intern. Cong. Biochem.*, **X28** (1970).
- Johnson, P., Harris, C. I., and Perry, S. V., *Biochem. J.*, **105**, 361 (1967).
- Weeds, A. G., *Nature*, **223**, 1362 (1969).
- Gazith, J., Himmelfarb, S., and Harrington, W. F., *J. Biol. Chem.*, **245**, 15 (1970).
- Schapiro, F., *Bull. Soc. Chim. Biol.*, **49**, 1647 (1967).

Effect of L-Asparaginase on Germinal Centre Haemolysin

THE immunosuppressive effects of L-asparaginase show an unusual time-dependence. In mice immunized with sheep red cells, asparaginase strongly inhibited the development of haemolytic plaque-forming cells in the spleen if given at any time from 24 h before to 3 h after administration of antigen; administration earlier or later was less effective, and even massive doses (5,000 IU/mouse or 250,000 IU/kg) had no significant effect if given 2 days after the antigen¹. These experiments suggested, therefore, that asparaginase did not exert its immunosuppressive effects by interfering with the rapid proliferation of immunocytes which is at its peak 2-3 days after the administration of antigen.

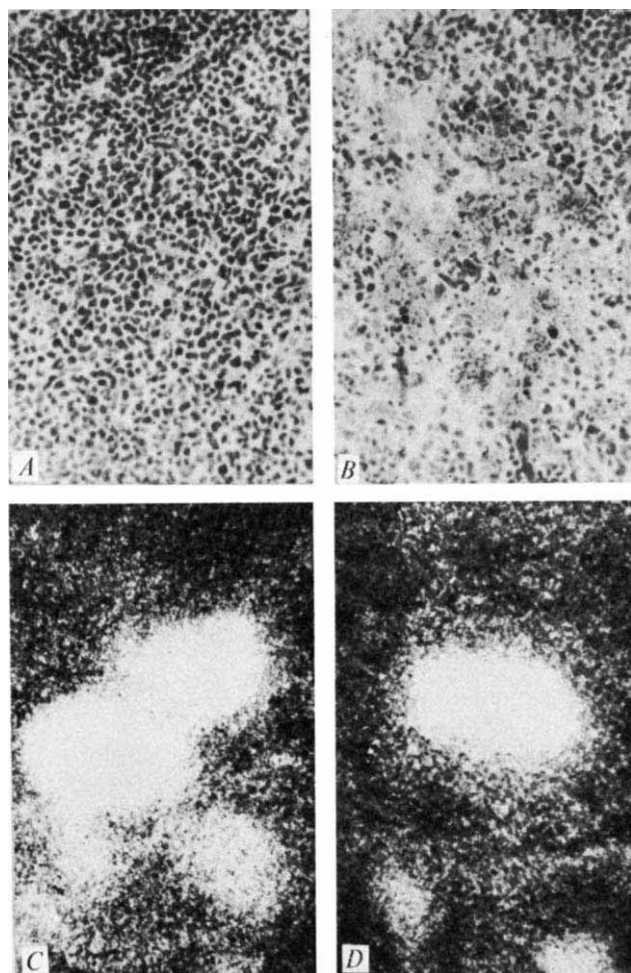


Fig. 1 Spleens of red cell-immunized mice injected with 2,000 IU of asparaginase (*B*, *D*) or saline (*A*, *C*). Spleens removed 4 h after injection, sectioned to show haemolytic areas and stained with haematoxylin and eosin³. *A*, *B*, Germinal centres, showing marked necrosis after asparaginase, $\times 200$. *C*, *D*, Haemolysis over germinal centres, $\times 22.5$. *C* and *D* were photographed on Ilford fine grain safety positive film 384, using a green filter (transmission 405–630 m μ , max 520 m μ).

Conflicting results were obtained when the effects of asparaginase were investigated in an *in vitro* system, in which rat lymphocytes react to mouse embryo cells by transforming and becoming actively lytic. Here it was found that proliferating transformed cells were rapidly destroyed by asparaginase².

Immunosuppressive doses of asparaginase cause necrosis of cells in the germinal centres of lymph nodes and spleen¹. These changes, which are maximal 4–8 h after injection, are transient, normal appearances being restored by 24 h. Germinal centres are conspicuous sites of haemolytic activity 6–12 days after injection of sheep red cells^{3,4}. It was, therefore, of interest to see whether the striking necrosis produced by asparaginase in these areas had any effect on their haemolytic activity.

Male Balb/C mice, weighing 18–24 g, were injected intraperitoneally with 0.2 ml. of a 10% suspension in saline of formalized sheep red cells (Burroughs Wellcome). Ten days later, they were given intraperitoneally 1,000 or 2,000 IU of L-asparaginase (Squibb) of *E. coli* origin, dissolved in saline. Control mice were immunized with red cells and given saline only, or were unimmunized and given asparaginase. At 4, 8 or 24 h after the asparaginase or saline injection, spleens were removed and frozen and 6 μ sections were cut in a cryostat and mounted on glass slides. The dried sections were coated with a suspension of sheep red cells in melted 0.7% agar dissolved in tissue culture medium 199, incubated in the presence

of guinea-pig complement, fixed, dried and stained. The method used was that described earlier³, with the modification that, to reduce non-specific lysis of red cells during fixation, this was carried out in formalin vapour overnight instead of in formalin solution.

Asparaginase caused the expected damage to germinal centres and, 4–8 h after giving 2,000 U/mouse, most cells in many centres formed necrotic, eosinophilic aggregates containing nuclear debris (Fig. 1*B*). Twenty-four hours after the injection, these masses had largely disappeared, presumably through phagocytosis, and the germinal centres were repopulated with cells of normal appearance. Neither at the time of maximal damage 4–8 h after giving asparaginase, nor at 24 h, was the haemolysis in mice treated with asparaginase distinguishable in distribution or intensity from that in mice given saline (Fig. 1*C* and *D*). Mice that had received asparaginase but not sheep red cells also showed germinal centre necrosis, but the necrotic areas were not haemolytic. This excludes the possibility that a reduction in immune haemolysin content following cell necrosis might have been obscured by the release of non-specifically lytic substances from dead cells.

It seems unlikely that necrotic cells containing antibody could retain it for more than a short time after their death, and the fact that normal haemolysis was observed over grossly damaged centres suggests that cells engaged in antibody production are not killed by asparaginase. The widespread cell death observed in germinal centres must therefore involve cells other than antibody-producers.

Although it is still possible that germinal centre haemolysins demonstrated 10 days after immunization are not actively being produced there but are adsorbed on to the surfaces of reticular or other cells in the germinal centres, it seems improbable that cell-adsorbed antibody which remains adsorbed in spite of the death of the cell will be able to diffuse into the overlying agar gel to cause haemolysis.

We thank the Cancer Research Campaign, the Leukaemia Research Fund, the Medical Research Council and the Nuffield Foundation for financial assistance, Professor R. M. Hardisty for asparaginase and Bernice McKelvie for assistance.

MORRIS C. BERENBAUM
SIDNEY BONDURANT*

*Wellcome Laboratories of Experimental Pathology,
Variety Club Research Wing,
St Mary's Hospital Medical School,
London W2*

Received November 4, 1970.

* Present address: University of California, Davis, School of Medicine, Sacramento.

¹ Berenbaum, M. C., *Nature*, **225**, 550 (1970).

² Berenbaum, M. C., Ginsburg, H., and Gilbert, D. M., *Nature*, **227**, 1146 (1970).

³ Berenbaum, M. C., and Stringer, I. M., *Immunology*, **18**, 85 (1970).

⁴ Young, I., and Friedman, H., in *Germinal Centres in Immune Responses* (edit. by Cottier, H.), 102 (Springer, Berlin, 1967).

Enhancement of Metastases by L-Asparaginase in a Mouse Tumour System

It has been reported that the new antileukaemic and anti-tumour agent, L-asparaginase, has significant immunosuppressive activity^{1–6}. We have studied the effect of L-asparaginase on the mouse tumour sarcoma 180 grown as a foot implant, which metastasizes only rarely to the regional popliteal node, but metastasizes extensively after the host has been treated with an immunosuppressive agent such as antilymphocyte serum⁷. There was a striking enhancement of metastases

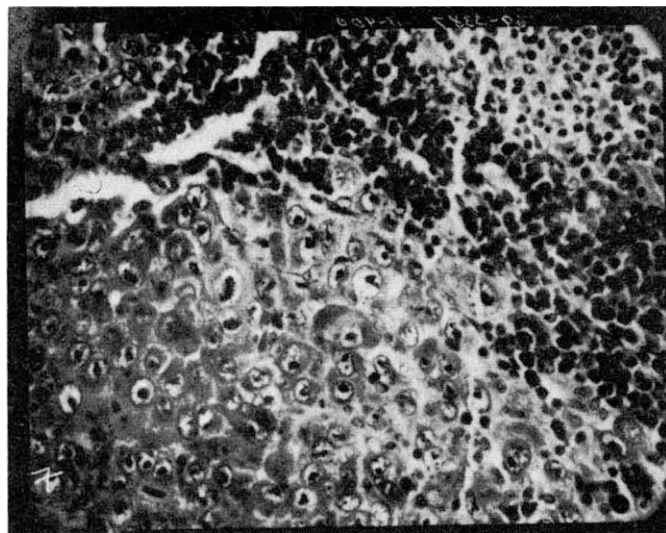


Fig. 1 Metastatic S180 tumour in a popliteal node from a mouse treated with L-asparaginase (10 IU/day). (Stained with haematoxylin-eosin, $\times 260$.)

when the mice were treated with L-asparaginase even in doses considerably smaller than those used by previous investigators which had no antitumour effect.

The tumour (originally obtained from Jackson Labs., Bar Harbor, and kept alive by serial transfer in C57BL/6 mice) was minced and suspended (1 : 2) with Hanks balanced salt solution (HBSS) and (0.02 ml.) was injected subcutaneously in each of the hind feet of C57 mice. The tumour was allowed to grow for 14 days, when the mice were killed and the right and left popliteal nodes were removed and examined microscopically. The lymph nodes were treated with Zenker's fixative and the sections stained with haematoxylin-eosin. Metastatic tumour was identified as sheets or clusters of tumour cells (five cells or more) in the subcapsular sinus of the lymph node (Fig. 1). The mice were divided into several groups, of ten or more. The negative control groups included untreated, normal mice, as well as those treated with normal rabbit serum, normal rabbit γ -globulin and human serum albumin. The positive control group included mice treated with rabbit antimouse thymus, antilymphocyte globulin (ALG 1 g%), prepared and administered as described previously⁸. The experimental groups included mice given intraperitoneal injections of L-asparaginase (a gift from Merck, Sharp and Dohme) given either daily from 4 days before to 10 days after implantation of the tumour or as a single injection on appropriate days as indicated. The popliteal lymph nodes of all the animals in each of the different groups were processed together for microscopic examination and, therefore, the results were expressed as proportion of total popliteal nodes rather than total animals showing metastases. This, however, does not alter the significance of our results.

The data shown in Table 1 for the various control groups represent our cumulative experience with this tumour system. In untreated normal mice the incidence of metastases from the foot tumour to the regional popliteal nodes was extremely small (4%). Treatment of these mice with various antigenic proteins such as rabbit γ -globulin, human serum albumin or normal rabbit serum did not enhance metastases. Similarly, physical manipulations of the primary tumour did not enhance the metastases. On the other hand, treatment of the mice with the potent immunosuppressive agent ALG led to a striking enhancement of metastases (94%). In mice given daily injections of L-asparaginase (Table 1) in doses ranging from 100–1 IU there was a similar striking enhancement of metastases. A small but significant effect was noted even with a dose of 0.1 IU daily and the dose had to be reduced to 0.01 IU before the effect became negligible. When L-asparaginase was given as a single injection in doses ranging from 500 to 10 IU at varying times in relation to the implantation of the primary tumour, the most

Table 1 Proportion of Popliteal Nodes with Metastases in Various Groups of Mice

No.	Group	Dosage per mouse per day	Proportion of nodes* with metastases (%)
1	Untreated, control	—	19/442 (4)
2	Treated with various proteins (normal rabbit serum, normal rabbit γ -globulin, human albumin)	1.0 mg	9/260 (4)
3	Physical manipulations of tumour (squeezing, ligation, needle puncture)	—	1/54 (2)
4	ALG (1 g%) treated positive control	0.1 ml.	205/219 (94)
5	L-Asparaginase	100 IU	36/40 (90)
6	L-Asparaginase	10 IU	84/90 (93)
7	L-Asparaginase	5 IU	30/33 (91)
8	L-Asparaginase	1 IU	32/58 (55)
9	L-Asparaginase	0.1 IU	4/20 (20)
10	L-Asparaginase	0.01 IU	1/20 (5)

Animals in groups 2, 4, and 5 to 10 received the appropriate agents daily starting from 4 days before the tumour implant to 10 days after implantation.

* Expressed in nearest whole number.

striking enhancement of metastases was noted when the agent was administered on the same day as the implantation of the tumour (Table 2). When the same dose of L-asparaginase was administered on day -4, day -2, day +2 or day +4 the effect was significantly less pronounced. There was no evidence of toxicity, haematological or systemic, of L-asparaginase when given in the doses used. Moreover, at this dosage there was no antitumour effect of this agent, for in all the experimental groups the primary tumours grew to the same size (tumour weight 400 to 500 mg as determined by subtracting the weight of normal foot from that of the tumour-bearing foot) as those in the various control group animals. The antitumour effect and the toxic effect of L-asparaginase were noted only at doses greater than 500 IU daily. When a daily dose of 1,000 IU was administered only half of the animals survived the experimental period and the average tumour weight was 15 mg.

Table 2 Effect of L-Asparaginase in a Single Dose on Enhancement of Metastases

Day of treatment	Proportion of nodes with metastases (%)— dosage per mouse			
	10 IU	50 IU	200 IU	500 IU
-4	—	3/20 (15)	4/18 (22)	5/20 (25)
-2	—	3/18 (17)	5/20 (25)	5/20 (25)
0 (day of tumour implant)	5/20 (25)	17/40 (43)	26/40 (65)	36/40 (90)
+2	—	2/20 (10)	5/18 (28)	7/20 (35)
+4	—	3/20 (15)	3/18 (17)	3/16 (18)

The tumour S180 behaves as an allogeneic graft in C57 mice and we have previously shown that the immunity in this system is primarily of the cellular type⁹. We have also demonstrated a definite relationship between immunosuppression of the host and the enhancement of metastases of the tumour. The only agents which have demonstrated significant enhancement of metastases in this system are also known to have a potent immunosuppressive action. Thus, in addition to ALG, these agents have included cyclophosphamide (cytoxan), prednisone and cytosine arabinoside¹⁰. The enhancing effect of L-asparaginase also is probably mediated through its immunosuppres-

sive effect. There are several interesting aspects of this effect. First, the doses of L-asparaginase causing significant enhancement of metastases were considerably smaller than those required for producing immunosuppression in other systems. Thus, in our experience and that of others¹⁻³, 10 IU or more of L-asparaginase per day per mouse was necessary significantly to inhibit the plaque-forming cell (p.f.c.) response of mice to injected sheep red blood cells or to produce significant enhancement of survival of skin allografts. Further, the doses of L-asparaginase which we used clearly had no inhibitory effect on the growth of the primary tumours perhaps indicating a greater sensitivity of the immune system (lymphocyte) to the action of this agent compared with that of the tumour cell. Second, when L-asparaginase was administered as a single dose, the enhancing effect was most striking when it was given on the day of tumour implantation. This suggests that L-asparaginase acts in the early, induction phase of the immune response.

To explore the possibility that L-asparaginase acts directly on the tumour cells in some unknown manner thereby enhancing their "metastatic potential", some preliminary experiments were carried out *in vitro* in which S180 cells (6×10^7 cells/ml.) were incubated *in vitro* at 37° C with equal volumes of L-asparaginase in varying concentrations for varying periods of time, followed by extensive washing of the tumour cells to remove L-asparaginase and finally injection of these cells in the subcutaneous tissue of the dorsum of the foot. When L-asparaginase was used in amounts up to 1,000 IU/ml. in such experiments, the primary tumours grew as usual and no enhancement of metastases to the regional popliteal nodes was noted. When more than 1,000 IU/ml. of L-asparaginase was used there was apparently a direct inhibitory effect on the tumour cells resulting in decreased growth (15 to 100 mg tumour weight) of the primary tumours; but here, too, no enhancement of metastases was noted.

The mechanism of action of L-asparaginase in its antitumour and immunosuppressive activity is generally believed to be in its ability to degrade asparagine which is required by tumour cells and presumably also by lymphocytes. In support of this hypothesis Chakrabarty and Friedman¹ demonstrated that simultaneous injection of asparagine with L-asparaginase prevented the immunosuppression when sheep red blood cells were used as antigen. In our mouse tumour system, however, administration of asparagine in daily doses as high as 10 mg and 20 mg per mouse did not decrease the incidence of metastases with the respective doses of L-asparaginase. The reasons for the discrepancy in these results are not clear.

This work was supported in part by grants from the Heart Association of North-east Ohio and Greater Cleveland Heart Fund.

SHARAD D. DEODHAR

Department of Immunology,
Divisions of Research and Laboratory Medicine,
The Cleveland Clinic Foundation,
Cleveland,
Ohio 44106

Received September 24; revised November 5, 1970.

- ¹ Chakrabarty, A. K., and Friedman, H., *Science*, **167**, 869 (1970).
- ² Schwartz, R., *Nature*, **224**, 275 (1969).
- ³ Nelson, S. D., Lee, M. B., and Bridges, J. M., *Transplantation*, **9**, 566 (1970).
- ⁴ Kahan, A., and Hill, J. M., *J. Immunol.*, **104**, 679 (1970).
- ⁵ Whitecar, J. P., Bodey, G. P., Harris, J. E., and Fioreich, E. J., *New Engl. J. Med.*, **282**, 732 (1970).
- ⁶ Ohno, R., and Hersh, E. M., *Blood*, **35**, 250 (1970).
- ⁷ Deodhar, S., and Crile, G., *Cancer Res.*, **29**, 776 (1969).
- ⁸ Deodhar, S., and Crile, G., *Lancet*, **i**, 168 (1968).
- ⁹ Deodhar, G., Crile, G., and Chiang, T., *Arch. Pathol.*, **90**, 79 (1970).
- ¹⁰ Deodhar, S., *Amer. J. Pathol.*, **59**, 98a (1970).

Tolerance to a Protein Antigen in a Poikilotherm, the Marine Toad *Bufo marinus*

IN spite of all the immunological studies which have been carried out with poikilothermic vertebrates, there have been no quantitative studies of the induction of tolerance to purified antigens, yet such studies might account for the difficulties often encountered in the immunization of lower vertebrates. Moreover, they should have a direct bearing on the phylogenetic emergence of the mechanisms involved in this crucial aspect of immunity. In this article, we shall show that adult marine toads (*Bufo marinus*) can be made tolerant to bovine serum albumin (BSA) by pretreatment with supraoptimal doses of the antigen.

Adult toads (National Reagents, Bridgeport, Connecticut) of both sexes (average weight about 250 g) were maintained at 37° C in moist sawdust and fed ground beef once a week. In the determination of the dose of BSA required to achieve optimal immunization, we gave the animals a single injection of antigen (Cohn Fraction V, Calbiochem, Los Angeles, California) in 0.15 M NaCl. Four groups of toads, each of eight to ten animals, were given doses of 0.25 mg, 10 mg, 25 mg and 250 mg of antigen, respectively, by intraperitoneal injection in 1.0 ml. of fluid. At weekly intervals after injection, the animals were anaesthetized with ether, and 1-2 ml. of blood was taken by cardiac puncture. Serum was prepared in the standard manner and antibody activity was assayed by passive haemagglutination¹. Control (sham) titrations using sheep red blood cells treated with the chromic chloride coupling agent but no antigen were always run in parallel with the assay with antigen coupled cells to check specificity. When no visible agglutination was observed at a dilution of 1:2, the titre ($\log_2$ of the reciprocal dilution at which definite agglutination was present) was defined to be zero.

Table 1 Relationship between Peak Titre and Antigen Dose for Immunization of the Toad with a Single Injection of Soluble BSA

Antigen dose (mg)	Specific passive haemagglutination titre *
0.25	2.9 ± 0.6
10.00	9.5 ± 1.2
25.00	5.5 ± 1.6
250.00	0.3 ± 0.3

Titres are expressed as specific titres, that is, $\log_2$ sham titres have been subtracted from the experimental titres for the same serum.

* Arithmetic mean ± s.d. Five to eight animals were used for each antigen dose.

The kinetics of the appearance of antibody in toad sera are very similar to those previously found for the response of these animals to bacterial flagella². There is a rapid increase in titre between the first and second weeks after immunization, and a plateau of maximum titre is reached by the third week. Table 1 illustrates the relationship between peak titre and dose, and it is seen that a maximum response was obtained by injection of 10 mg BSA. There was a slightly reduced response with 25 mg. Injection of 0.25 mg BSA evoked a definite but slight response but at the other extreme no significant titre was found for the group of toads given 250 mg of BSA.

We used an experimental design similar to that of Mitchison³ to tell whether the lack of response to large doses of BSA indicates a specific immunosuppressive effect. Each of eight toads was given ten injections each consisting of 200 mg of BSA in saline over a period of 3 weeks. Eight toads of a second group were each given ten injections of saline during the same period. Four days after the final injection of the pretreatment series, each animal from both sets was given a mixed injection containing 10 mg of BSA and 1 mg of human IgG (Cohn

Fraction II, Lederle Laboratories, Pearl River, New York) in complete Freund's adjuvant. Table 2 presents data obtained 5 and 6 weeks after antigenic challenge. The passive haemagglutination titres to BSA were clearly suppressed in the BSA-pretreated group. They were not significantly different from the sham titres. By contrast, no significant differences were observed between the titres to human IgG in the experimental and control groups.

In order to show that the cause of this specific decrease in titre was not merely the presence of free BSA in the serum masking antibody which might have been produced, we studied clearance of radioactively labelled BSA by control toads and animals given a suppressive dose of BSA. This protein was radio-iodinated to a specific activity of 0.6 $\mu\text{Ci}/\mu\text{g}$ with ^{125}I -iodide by means of a modification⁴ of the method of Greenwood *et al.*⁵.

Table 2 Effect of Pretreatment with Large Doses of Soluble Bovine Serum Albumin (BSA) on the Antibody Titres to Homologous and Heterologous Antigens in Adjuvant

Group	Antigen used to coat cells	Weeks after challenge	Titre*	Values compared†	Level of significance
Exp.	1-BSA	5	2.6 ± 0.5	1 and 7	1%
	2-IgG		6.6 ± 0.9		
	3-sham		2.8 ± 0.6	1 and 3	NS
	4-BSA	6	2.4 ± 0.5	4 and 10	1%
	5-IgG		6.5 ± 0.6		
	6-sham		2.6 ± 1.0	4 and 6	NS
	7-BSA	5	5.9 ± 2.0	7 and 9	1%
	8-IgG		7.9 ± 2.0	8 and 2	NS
	9-sham		3.3 ± 0.9		
Control	10-BSA	6	5.6 ± 2.1	10 and 12	1%
	11-IgG		7.5 ± 1.8	11 and 5	NS
	12-sham		1.8 ± 0.8		

NS, not significant; Exp, toads given 200 mg of BSA $\times$ 10, then a mixed challenge consisting of both BSA and IgG in adjuvant. Controls were given saline $\times$ 10, then a mixed challenge as above.

* Log_2 passive haemagglutination titre $\pm$ sample standard deviation.

† Statistical comparison of the titres corresponding to the given numbers was made using the *t* test and Bessel's correction for the standard error of the mean for small samples.

Seven experimental toads were each given one injection of 250 mg of BSA in saline. Seven control animals were each given one injection of diluent. Eight days afterwards, each toad was given a challenge injection in saline of 10 mg of BSA containing 20 μCi of ^{125}I -BSA. The animals were bled daily for 2 weeks and again on day 22, when they were killed. The results of this experiment are shown in Fig. 1 which gives the kinetics of clearance of radioiodinated BSA by both groups. Regression lines were fitted to the data by the method of least squares. The control group gave a classical picture⁶ of non-immune catabolic elimination ($t=1/2$ approximately 8 days) until about days 9 to 10 when immune clearance became obvious by the sharp change in slope. By contrast, only metabolic clearance was observed in the group pretreated with soluble BSA. Furthermore, the control group possessed passive haemagglutination titres of 6.4 ± 0.9 at day 22, whereas the experimental group exhibited no detectable titre. The amount of ^{125}I -BSA remaining in the control group after 22 days was not statistically different from background level.

The present results add further support to the conclusion^{2,7-9} that the basic immunological mechanisms of mammals emerged by the phylogenetic level of higher amphibians. We found that large doses of BSA suppressed the formation of antibodies to

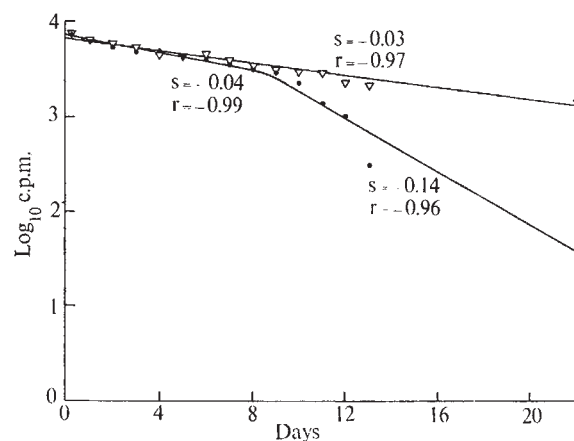


Fig. 1 Elimination of ^{125}I -BSA by normal and immunologically suppressed toads. ●, Arithmetic mean of c.p.m. in 5 μl of serum from seven toads given a single injection of saline prior to injection of 10 mg of BSA. ▽, Arithmetic mean of c.p.m. in 5 μl of serum from seven toads given a single injection of 250 mg of BSA in saline before challenge with 10 mg of the same antigen. The antigenic challenge of 10 mg of BSA given to both groups of animals also contained 20 μCi of ^{125}I -BSA. Regression lines were fitted to the data by the method of least squares. *r*, Correlation coefficient. *s*, Slope. Two slopes are given for the curve obtained with the saline-injected control animals: the first is computed for days 2-9; the second slope corresponds to days 10-22 inclusive. The slope of the line for the BSA-treated animals applies to the curve from days 2-22 inclusive.

this antigen, but did not affect antibody-production to an unrelated protein, human IgG. In a comparative context, it is interesting to note that the doses of soluble BSA required to induce immunity and high zone tolerance in toads (5 $\mu\text{g/g}$ body weight and 1 mg/g body weight, respectively) corresponded closely to those values observed in mice for the same antigen³. Since the cellular mechanisms of antigen retention^{2,9} and antibody-production^{2,7,8} in anurans have been shown to be basically similar to the corresponding processes in mice, it is reasonable to presume that the cellular mechanisms responsible for induction of high zone tolerance are also similar in these phylogenetically diverse groups.

This work was supported by a research grant from the US National Institute of Allergy and Infectious Diseases. We thank Mrs Maria Devlin for technical assistance. Reprint requests should be sent to John J. Marchalonis at the Walter and Eliza Hall Institute of Medical Research, Post Office, The Royal Melbourne Hospital, Victoria 3050, Australia.

JOHN J. MARCHALONIS
RONALD N. GERMAIN

Division of Biological and Medical Sciences,
Brown University,
Providence,
Rhode Island 02912

Received July 13; revised October 27, 1970.

¹ Gold, E. R., and Fudenberg, H. H., *J. Immunol.*, **99**, 859 (1967).

² Diener, E., and Marchalonis, J. J., *Immunol.*, **18**, 279 (1970).

³ Mitchison, N. A., *Proc. Roy. Soc., B*, **161**, 275 (1964).

⁴ McConahey, P. J., and Dixon, F. J., *Intern. Arch. Allergy*, **29**, 185 (1966).

⁵ Greenwood, F. C., Hunter, W. M., and Glover, J. S., *Biochem. J.*, **89**, 114 (1963).

⁶ Talmage, D. W., Dixon, F. J., Bukantz, S. C., and Dammin, G. J., *J. Immunol.*, **67**, 243 (1951).

⁷ Marchalonis, J. J., and Edelman, G. M., *J. Exp. Med.*, **124**, 901 (1966).

⁸ Marchalonis, J. J., Allen, R. B., and Saarni, E. S., *Comp. Biochem. Physiol.*, **35**, 49 (1970).

⁹ Pollara, B., Cain, W. A., Finstad, J., and Good, R. A., in *Biology of Amphibian Tumours* (edit. by Mizell, M.), 169 (Springer New York, 1969).

Evidence that Piroplasms can undergo Antigenic Variation

In many babesia and malaria infections, the host carries a persistent low level parasitaemia after recovery from the acute phase of the disease, and splenectomy of such chronically infected animals usually precipitates a parasitic recrudescence which can be fatal. During chronic infections by *Plasmodium knowlesi* in rhesus monkeys, the parasite undergoes repeated antigenic variation¹, and splenectomy of a chronically infected monkey precipitates an acute parasitaemia, the parasites of which are antigenically different from those with which the monkey was initially infected. Repeated antigenic variation is, however, not yet known to be a characteristic of most malaria or of any babesia infections. We compared the antigenic character of two populations of the piroplasm, *Babesia rodhaini*, one population being used to initially infect a rat and the second isolated from the same rat when the infection had become subpatent.

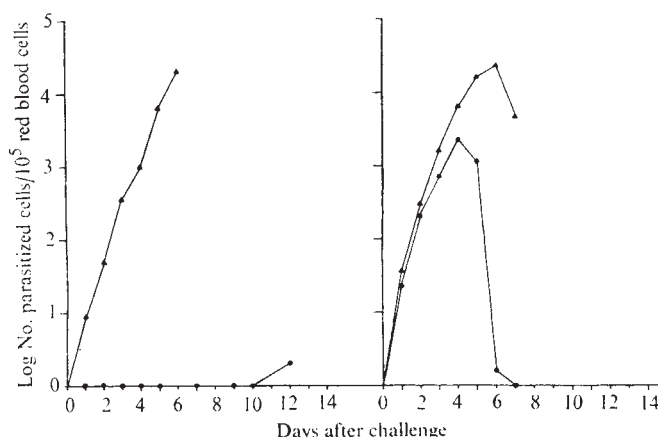


Fig. 1 Rats, immunized with population *N* (infecting population) of *B. rodhaini*, were challenged with 4×10^7 parasitized red cells of either population *N* (left) or *R* (subpatent population) (right). The parasitaemias in the figure are the geometrical means of five or six rats. ●, Controls; ▲, immunized.

The strain of *B. rodhaini* was rat-adapted and free from *Eperythrozoon coccoides*. Male Sprague-Dawley rats, bred at this institute, were used. Reference stabilates² of the parasitic populations were stored at -70°C . The technique of immunizing rats with irradiated piroplasms has been described³; parasitized or normal rat red cells received 50 krad from a γ -beam ^{60}Co source delivered at a dose rate of $40,000\text{ r min}^{-1}$. All injections were given intraperitoneally and parasitaemias were assessed by examining Giemsa-stained tail blood smears.

An experiment typical of several is described here, and a more detailed account of this work is in preparation. A 120 g rat was infected with a reference population of *B. rodhaini*, designated population *N*. The rat recovered from the acute parasitaemia which ensued and 2 days after the parasitaemia had become subpatent, the rat was splenectomized. Fifteen days later a parasitic recrudescence became patent in this rat and from this recrudescence a population (*R*) was frozen. Populations *N* and *R* were then compared by examining the response of rats immunized with population *N* to challenge from either *N* or *R* populations as follows.

Two groups of 60–80 g rats were immunized by injecting approximately 4×10^9 irradiated parasitized cells of population *N* on two occasions 6 days apart. Two groups of control rats received irradiated, uninfected red cells. Two weeks after the last immunizing injection, one group of immunized and one group of control rats were challenged with 4×10^7 parasitized cells of population *N*, and one group of immunized and one of controls with 4×10^7 parasitized cells of population *R*. The

response to these challenges is given in Fig. 1. Rats immunized with *N* and challenged with *N* parasites showed no parasites for 12 days, whereas the corresponding controls suffered acute parasitaemias, which were patent on the day after challenge. In contrast, both the *N*-immunized rats and the unimmunized controls challenged with *R* parasites showed parasites at the same time: during the first 4 days of the infection their parasitaemias developed similarly, but the immunized rats then developed a protective response, 2 days before the controls. This and other similar results suggest that the populations of piroplasms isolated from recovered rats following splenectomy are antigenically different from that originally used to infect the rats. Sensitization of rats to one population, however, enabled them to mount an anamnestic-type response to a "variant" population.

Thus *B. rodhaini* undergoes antigenic variation during the course of an infection. It is probably by making this type of change that this and most other piroplasms can continue to multiply in an apparently immune host. Therefore immunological studies on piroplasms, investigations into the epidemiology of piroplasmosis and the development and application of a piroplasm vaccine must take antigenic variation into account.

The technique described here for detecting apparent antigenic variation in *B. rodhaini* infections could be used to follow antigenic variation in other protozoal infections.

R. S. PHILLIPS

National Institute for Medical Research,
Mill Hill,
London NW7

Received January 8; revised March 10, 1971.

¹ Brown, K. N., and Brown, I. N., *Nature*, **208**, 1286 (1965).

² Lumsden, W. H. R., and Hardy, G. T. C., *Nature*, **205**, 1032 (1965).

³ Phillips, R. S., *Nature*, **227**, 1255 (1970).

Absence of Enhancing Antibody in Cell Mediated Immunity to Tumour Heterografts in Protein Deficient Rats

The classical association between famine and pestilence reflects the decrease in host resistance to bacterial infection occurring in malnutrition¹. Little is known, however, about the influence of malnutrition on cell-mediated immunity². A marked decrease in incidence and growth of spontaneous and transplanted malignancies in animals fed restricted diets, first reported by Moersch³ and Rous⁴, has been confirmed for many tumour types in rodents^{5–7} and cattle⁸ and with restriction of either caloric⁹ or protein intake¹⁰. Malnutrition increases resistance to virus infections in man¹¹.

We have observed depressed antibody responses and increased peripheral blood lymphocyte transformation to influenza A₂ antigen in malnourished Australian Aboriginal children following vaccination¹². Protein deficient mice in our laboratory responded normally to phytohaemagglutinin and rejected skin allografts more rapidly than normally nourished controls while showing striking depression of antibody synthesis to sheep red blood cells¹³. The apparent increase in cellular immunity in malnutrition suggested by these studies may be related to increased host resistance to malignancy and virus infection.

Our study was designed to assess cellular and humoral immunity to mouse tumour cells in rats with different degrees of protein-calorie deprivation. Outbred weanling Charles River male rats, of mean weight 84 g, were divided into three equal groups matched by body weight. Group 1 animals received an *ad libitum* diet of 30% casein, 30% dextrose, 30% cornstarch, 4% corn oil, 4% salt mixture XIV, 0.5% vitamin

mixture and 1.5% agar (Nutritional Biochemicals). Group 2 animals received *ad libitum* a low protein diet of 8% casein, 41% dextrose, 41% cornstarch with other additives as for Group 1. Group 3 received a low calorie diet of 22% casein, 30% dextrose, 30% cornstarch, 8% corn oil, 8% salt mixture, 1% vitamin mixture and 1% agar but with total daily quantity reduced to half the dry weight of food consumed by group 2. Diets were started when the animals were 3 weeks old, and they were immunized at approximately 12 weeks of age, when group 1 rats weighed 654 (s.d. ± 13) g, group 2 rats weighed 226 (± 61) g and group 3 rats weighed 177 (± 23) g. Rats received a single intraperitoneal immunizing dose of 10^5 viable DBA/2 mouse mastocytoma P-815-X2 ascites tumour cells per g body weight (1.5×10^7 to 5×10^7 cells per rat). Spleen cells and serum were obtained from one normal unimmunized animal and from matched rats in each nutritional group 11 days after immunization. *In vitro* tests of cellular immunity were performed by the method of Brunner¹⁴ with minor modification. Mastocytoma target cells cultured *in vitro* were labelled for 45 min at 37° C with 10% sodium chromate labelled with ⁵¹Cr (1 mCi/ml., specific activity 200–500 Ci/ μ g chromium) washed five times, adjusted to 2×10^5 viable cells per ml. and mixed with 2×10^7 viable spleen cells per ml. in aliquots of 0.5 ml. of Dulbecco's medium and 10% inactivated calf serum. Target cell lysis was measured after 4 h by comparison of supernatant radioactivity in mixtures of target cells with sensitized and non-sensitized lymphocytes. Serum blocking activity was determined by substituting 10% inactivated rat serum for calf serum in reaction mixtures. Serum cytotoxic activity was measured by incubating aliquots of target cells with 10% inactivated rat serum to which 0.1 ml. guinea-pig complement was added 30 min before termination at 4 h. Total releasable radioactivity was determined by incubation of one aliquot of target cells with 2 ml. of distilled water for 4 h. Radioactivity was measured in a well-type gamma counter (Nuclear Chicago). All experiments were performed in triplicate.

Table 1 Lysis of ⁵¹Cr-labelled Mastocytoma Target (T) Cells by Sensitized Spleen Lymphoid (L) Cells after 4 h Incubation and Effect of Serum from Three Nutritional Groups

Diet	T cells, 10% calf serum; L cells from various groups		Serum from different groups; T cells with L cells from group 1 animals	
	c.p.m. *	% Specific lysis †	c.p.m.	% Specific lysis
Group 1 30% protein	3,398 $\pm$ 285	62	970 $\pm$ 282	5
Group 2 8% protein	3,650 $\pm$ 343	68	3,667 $\pm$ 206	67
Group 3 22% protein half caloric	3,194 $\pm$ 224	57	1,519 $\pm$ 182	17
Non-immunized normal animal	781 $\pm$ 132	0	3,573 $\pm$ 271	66

* Mean c.p.m. $\pm$ s.d. of ten experiments after adjustment of maximum releasable radioactivity in each test to 5,000 c.p.m.

† c.p.m. of specimen – c.p.m. with non-immunized lymphocytes

Total releasable activity – c.p.m. with non-immunized lymphocytes $\times 100\%$.

Spleen lymphocytes from immunized animals in all nutritional groups were almost equally active in target cell lysis (Table 1). Addition of inactivated serum from sensitized animals on 30% protein diets markedly inhibited cell mediated lysis in seven experiments and showed less than 50% inhibition in three experiments. Serum from sensitized protein deficient

animals showed no inhibition of cell mediated lysis in any of ten experiments, even after pre-incubation of target cells in undiluted serum. Incubation of both lymphoid cells and serum from protein deficient animals produced ten-fold greater lysis of target cells than with cells and serum from immunized normal animals. Target cell lysis was markedly increased in 10% inactivated serum from normally nourished rats (Table 2) after the addition of complement.

Table 2 Serum Mediated Specific Lysis of ⁵¹Cr-labelled Mastocytoma Target (T) Cells with and without Added Complement

Diet	T cells with inactivated serum from various groups		T cells with inactivated serum from various groups with complement added	
	c.p.m.	% Specific lysis	c.p.m.	% Specific lysis
Group 1 30% protein	1,552 $\pm$ 132	16	4,261 $\pm$ 372	82
Group 2 8% protein	1,347 $\pm$ 87	11	1,922 $\pm$ 143	25
Group 3 22% protein half caloric	1,511 $\pm$ 123	15	2,578 $\pm$ 196	41
Unimmunized control	896 $\pm$ 96	0	1,675 $\pm$ 121	19

The site of action of blocking serum factors was determined by incubation of labelled target cells for 30 min at 37° C with 10% inactivated serum known to block cell mediated immunity. After two washings the target cells were mixed with sensitized lymphocytes in complete media and 10% inactivated calf serum and specific lysis determined. Sensitized spleen lymphoid cells were also pre-incubated with known blocking serum, washed and mixed with aliquots of labelled target cells (Table 3). Blocking activity of serum was markedly reduced after incubation for 4 h with two changes of packed DBA/2 spleen cells or DBA/2 mastocytoma, but not following incubation with C57B1 mouse spleen cells or normal rat spleen cells.

Table 3 Effect of Pre-incubation of ⁵¹Cr-labelled Mastocytoma Target (T) Cells or Alternatively Sensitized Lymphoid (L) Cells from Group 1 Animals with Serum from Sensitized Animals in Various Nutritional Groups

Diets	T cells pre-incubated		L cells pre-incubated	
	c.p.m.	% Specific lysis	c.p.m.	% Specific lysis
Group 1 30% protein	1,591 $\pm$ 178	21	2,497 $\pm$ 378	42
Group 2 8% protein	3,015 $\pm$ 271	54	3,185 $\pm$ 401	58
Group 3 22% protein half caloric	2,238 $\pm$ 275	36	2,755 $\pm$ 418	48
Non-immunized normal control	3,187 $\pm$ 294	58	3,360 $\pm$ 287	62

Specific lysis was measured after washing and testing in standard reaction mixture.

We have confirmed in a heterozygous system the development of cytotoxic cellular immunity and specific serum inhibition of cellular immunity against DBA/2 mouse mastocytoma transplantation antigens, previously reported by Brunner¹⁴ in the mouse. That this sensitive *in vitro* cytotoxic assay measures thymic dependent cellular immunity was shown by inhibition in the mouse with sera of antitheta specificity¹⁵ but not by sera of anti-immunoglobulin heavy chain specificity or com-

plement inhibitors¹⁶. Specific serum factors which block cellular immunity *in vitro* and enhance specific tumour growth *in vivo* are considered to be gamma globulins¹⁷ with major activity in 7S gamma globulin fractions¹⁸ and in the mouse possibly IgG gamma₂ (ref. 19). Our findings suggest that, at this level of moderate chronic protein or caloric deficiency, specific cell mediated immunity to transplantation antigens develops normally. Complement-dependent cytotoxic antibody and blocking antibody production to cellular antigens are markedly depressed in protein deficient animals^{20,21}. The reported failure to demonstrate depression of antibody production in malnourished animals to bacterial and soluble antigens requires further analysis¹³. The apparent increase in cellular immunity in malnourished animals^{12,13} may be due to the failure of nutritionally deficient animals to develop a specific antibody which in the normal animal blocks cellular immunity. The immune surveillance mechanism for the elimination of malignant and virus-infected cells may operate more efficiently in moderate malnutrition and provide one of several adverse factors limiting growth and replication of aberrant cells in animals with nutritional deprivation.

Cell mediated immunity directed against tumour specific antigens and specific antibody blocking cellular cytotoxic immunity *in vitro* both occur with many mammalian tumours^{17,22} including human neuroblastoma and adenocarcinoma of the lung and colon²³. Specific antibody may block cellular immunity by competing with sensitized lymphocytes for the same antigenic determinants at the target cell surface¹⁸, or by directly inhibiting the sensitized lymphoid cell¹⁷. Both mechanisms were found to operate in the tumour heterograft system used in this study. Greater exposure of sensitized lymphoid cells to specific antibody in the normally nourished animal *in vivo* may explain slight increases in lysis by cells from protein deficient animals in this study and the increase in lymphoblast transformation to specific antigen *in vitro* found previously in malnourished Aboriginal children¹².

A major barrier to active immunotherapy of malignancy has been the stimulation of enhancing antibody¹⁷. In the rat the development of enhancing antibody can be depressed while maintaining cell mediated immunity intact by feeding a diet of 8% casein, which provides less than the basal daily requirement of the essential sulphur containing amino-acids methionine and cystine. It seems possible by nutritional or metabolic manipulation that such a mechanism could open the way to successful immunotherapy of human tumours.

This work was supported by grants from the American Cancer Society, the American Heart Association, the National Foundation—March of Dimes and the US Public Health Service. D. G. J. was supported by a C. J. Martin Fellowship of the National Health and Medical Research Council of Australia.

DAVID G. JOSE
ROBERT A. GOOD

Departments of Pediatrics and Pathology,
Variety Club Heart Hospital,
Minneapolis,
Minnesota 55455

Received November 2, 1970; revised March 2, 1971.

- ¹ Dubos, R., *Man Adapting* (Yale University Press, 1965).
- ² Good, R. A., Finstad, J., and Gatti, R. A., in *Infectious Agents and Host Reactions* (edit. by Mudd, S.), 105 (Saunders, Philadelphia, 1969).
- ³ Moreschi, C., *Z. Immunitätsforsch.*, ii, 651 (1909).
- ⁴ Rous, P., *J. Exp. Med.*, 20, 433 (1914).
- ⁵ White, J., and Andervont, H. B., *J. Nat. Cancer Inst.*, 3, 499 (1943).
- ⁶ Saxton, J. A., Boon, M. C., and Furth, J., *Cancer Res.*, 4, 401 (1944).
- ⁷ Ross, M. H., and Bras, G., *J. Nutr.*, 87, 245 (1965).
- ⁸ Anderson, D. E., Pope, L. S., and Stephens, D., *J. Nat. Cancer Inst.*, 45, 697 (1970).
- ⁹ Tannenbaum, A., and Silverstone, H., *Adv. Cancer Res.*, 1, 451 (1953).
- ¹⁰ Theuer, R. C., *J. Nutr.*, 101, 223 (1971).

- ¹¹ Scrimshaw, N. S., Taylor, C. E., and Gordon, J. E., *Amer. J. Med. Sci.*, 237, 367 (1959).
- ¹² Jose, D. G., Welch, J. S., and Doherty, R. L., *Austral. Paediat. J.*, 6, 192 (1970).
- ¹³ Cooper, W. C., Mariani, T., and Good, R. A., *Fed. Proc.*, 29, 364 (1970).
- ¹⁴ Brunner, K. T., Maud, J., Rudolf, M., and Chapuis, B., *Immunology*, 18, 501 (1970).
- ¹⁵ Cerottini, J. C., Nordin, A. A., and Brunner, K. T., *Nature*, 228, 1308 (1970).
- ¹⁶ Canty, T. G., and Wunderlich, J. R., *J. Nat. Cancer Inst.*, 45, 761 (1970).
- ¹⁷ Kaliss, N., *Transplant. Proc.*, 2, 59 (1970).
- ¹⁸ Brunner, K. T., Maud, J., Cerottini, J. C., and Chapuis, B., *Immunology*, 14, 181 (1968).
- ¹⁹ Takasugi, M., and Hildemann, W. H., *J. Nat. Cancer Inst.*, 43, 857 (1969).
- ²⁰ La Via, M. F., Barker, P. A., and Wissler, R. W., *J. Lab. Clin. Med.*, 48, 237 (1956).
- ²¹ Kenney, M. A., Roderick, C. E., Arnrich, L., and Piedad, F., *J. Nutr.*, 95, 173 (1968).
- ²² Billingham, R. E., Brent, L., and Medawar, P. B., *Transplant. Bull.*, 3, 84 (1956).
- ²³ Hellstrom, I., Hellstrom, K. E., Evans, C. A., Heppner, G. M., Pierce, G. E., and Yang, J. P. S., *Proc. US Nat. Acad. Sci.*, 62, 362 (1969).

Evidence for Temporary Slowing of Mucociliary Clearance in the Lung caused by Tobacco Smoking

IN previous reports^{1,2} from this laboratory no evidence was found of irreversible impairment of mucociliary clearance in humans as the result of prolonged tobacco smoking. This does not exclude the possibility that temporary slowing occurs after each smoke, followed by recovery between smokes or overnight. Although this has not been demonstrated previously in humans, many experiments on animals^{3,4} or with excised epithelium^{5,6} have shown reversible slowing of ciliary activity on exposure to tobacco smoke. In postulating a relation between ciliary stasis and lung cancer, Hilding⁷ had in mind irreversible damage, but even temporary slowing of the mucous flow would increase the exposure of bronchial epithelium to inhaled noxious substances and carcinogens, thereby increasing the likelihood of bronchitis and carcinoma of the lung.

We describe here a study of the acute effects of smoking on mucociliary clearance using methods already reported^{1,8}. In brief, clearance was assessed by monitoring, externally to the chest, the removal of 5 μ m particles labelled with technetium-99m⁹ which had been inhaled in controlled conditions. (This procedure has been approved by the Medical Research Council's Isotope Panel, the Ethical Subcommittee of this School and the Atomic Energy Research Establishment, Harwell, for participation by their own staff¹⁰.)

Twenty-two volunteers (aged 64–92; mean 74.9) from old people's homes were examined, of whom nine were female. All were current smokers except for one female. Previous experience and the present study indicated that there was no sex difference in rate of clearance so the females have been grouped with the males throughout our report. The twenty-one smokers comprised nineteen cigarette smokers (mean and range of pack-years, 19,200; 3,100–71,200) and two pipe smokers. Ventilatory capacity tests performed on all subjects revealed that in the study group eight subjects, of whom three were female, had mild restrictive impairment and two males had airway obstruction¹, but all were ambulant and "fit".

Lung clearance curves obtained by the method used are usually exponential^{11,12}, probably because ciliary streaming is much faster in the larger than the finer airways¹³. In this study gamma-ray counting was done at frequent intervals for 6 h commencing immediately after inhalation of the tracer particles. The counts were corrected for background and radioactive decay and were plotted as soon as read until such time as the slope of the curve appeared to be established. Subjects were then asked to smoke several cigarettes (two to five)

Table 1 The Effect of Smoking on Rates of Mucociliary Clearance *

	No. of subjects	Means and s.e. of the clearance rates			Paired <i>t</i> values	No. of individuals with significant difference in clearance rates at 5% level
		Before smoking	During smoking	Difference		
Total	22	-0.0161 (±0.0035)	-0.0078 (±0.0022)	-0.0084 (±0.0036)	2.360†	10 (45.5%)
Normal	12	-0.0112 (±0.0032)	-0.0081 (±0.0033)	-0.0033 (±0.0049)	0.700	4 (33.3%)
Restrictive	8	-0.0259 (±0.0074)	-0.0074 (±0.0038)	-0.0185 (±0.0054)	3.532‡	6 (75.0%)
Obstructive	2	-0.0062 (±0.0017)	-0.0077 (±0.0052)	+0.0015 (±0.0033)	—	0

* $\ln$ (% deposition-asymptote)/min.† $P < 0.05$.‡ $P < 0.01$.

consecutively without constraint as to time. Care was taken to ensure that the subjects did not smoke for at least 1 h before the tests.

Change in slope has been evaluated by comparing the calculated regressions of that portion of the curve from the first count to the beginning of smoking with that from the beginning of smoking to the end of the smoking period. To eliminate curvature before examining the curve for smoking effects the most appropriate transformation is

$$z_t = \ln(y_t - \text{asymptote})$$

where y_t is the percentage of the initial lung burden present at any time t . The rate of clearance will henceforth be expressed in terms of z_t . As an estimate of the asymptote we have taken 95% of the smallest value recorded in the 6 h interval.

Table 1 shows the effect of smoking on the clearance curves. The mean difference (-0.0084) between the slopes of the regression lines for the pre-smoking (-0.0161) and smoking (-0.0078) periods for the whole group of twenty-two subjects was significant at the 5% level. The difference in the slopes was normally distributed. Subjects with restrictive impairment had on the average a larger difference than the normal group as the result of smoking (-0.0185 against -0.0033). This was due chiefly to the steeper pre-smoking clearance rate, since the mean for the smoking period was nearly the same in these two groups. Student's t tests on the slopes of individual subjects before and during smoking showed that changes in slopes were significant in four out of twelve normals and in six out of eight "restrictive" subjects.

Other factors which might have affected the change in slope are: (1) previous smoking history; (2) whether or not the subjects were accustomed to inhale the smoke; and (3) variables associated with type¹⁴ and quantity of cigarettes smoked during the experiment. Of the ten individuals who had a significant change in the slope nine were cigarette smokers who gave a history of inhaling cigarettes; the remaining individual was one of the two pipe smokers who, however, smoked cigarettes during the experiment. A further six inhalers did not give significant changes in slope ($\chi^2 = 2.4$; $P < 0.2$). The average product of tobacco consumption and the time of smoking was 109 g-min for the ten subjects who showed a significant change in the slopes as compared to 58 g-min for the twelve subjects with non-significant change ($t = 2.16$; $P < 0.05$). We feel that these results require confirmation and hope to extend this investigation.

We thank the Tobacco Research Council for a grant, Mrs S. M. Forsey for technical assistance and the volunteer subjects.

D. PAVIA
M. L. THOMSON
S. J. POCKOCK

TUC Centenary Institute of Occupational Health,
London School of Hygiene and Tropical Medicine,
Keppel Street,
London WC1E 7HT

Received January 18, 1971.

- ¹ Pavia, D., Short, M. D., and Thomson, M. L., *Nature*, **226**, 1228 (1970).
- ² Pavia, D., and Thomson, M. L., *Lancet*, ii, 101 (1970).
- ³ Carson, S., Goldhamer, R., and Carpenter, R., *Amer. Rev. Respirat. Dis.*, **93**, 86 (1966).
- ⁴ Kaminski, E. J., Fancher, O. E., and Calandra, J. C., *Arch. Environ. Health*, **16**, 188 (1968).
- ⁵ Kensler, C. J., and Battista, S. P., *Amer. Rev. Respirat. Dis.*, **93**, 93 (1966).
- ⁶ Kensler, C. J., and Battista, S. P., *Fed. Proc.*, **23**, 105 (1964).
- ⁷ Hilding, A. C., *New Engl. J. Med.*, **256**, 634 (1957).
- ⁸ Few, J. D., Short, M. D., and Thomson, M. L., *Radiochem. Radioanal. Lett.*, **5**, 275 (1970).
- ⁹ *Lancet*, i, 131 (1968).
- ¹⁰ Booker, D. V., Chamberlain, A. C., Rundo, J., Muir, D. C. F., and Thomson, M. L., *Nature*, **215**, 30 (1967).
- ¹¹ Holma, B., in *Inhaled Particles and Vapours*, **2**, 189 (Pergamon, London, 1967).
- ¹² Morrow, P. E., Gibb, F. R., and Gazioglu, K., in *Inhaled Particles and Vapours*, **2**, 351 (Pergamon, London, 1967).
- ¹³ Hilding, A. C., *Arch. Environ. Health*, **6**, 61 (1963).
- ¹⁴ Dalhamn, T., and Rylander, R., *Nature*, **201**, 401 (1963).

Quinacrine Fluorescence in Mammalian Chromosomes

SINCE the possibilities of revealing chromosomal structure by staining chromatin with dyes derived from acridine first became apparent¹, we have used the antimalarial compound quinacrine dihydrochloride ('Atebrin', G. Gurr Ltd) to study the highly fluorescent area on the long arms of the human *Y* chromosome and in localized regions of certain autosomes²⁻⁴. We now describe an investigation of the chromosomes of various mam-

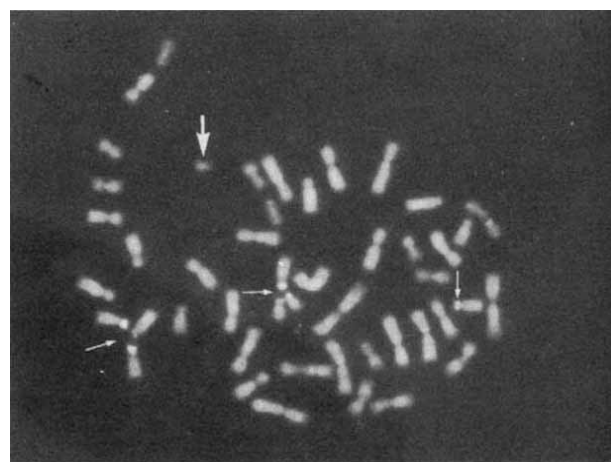


Fig. 1 Human metaphase with an intensely fluorescent *Y* chromosome (large arrow) and intensely fluorescent autosomes (small arrows).

mals, including man, to see whether quinacrine fluorescence is a universal property of all mammalian Y chromosomes or a reaction specific to man. We find that fluorescence of the intensity found on the human Y chromosome is only present in the African great apes and man.

Venous blood from the mammals was put into a standard microblood lymphocyte culture routinely used for human chromosome work⁵. The slides were stained for 5 min in a 0.5% aqueous solution of 'Atebrin', briefly rinsed in tap water, dried gently over a bunsen flame and mounted in distilled water of about pH 5.5. The coverslips were ringed with rubber cement to prevent drying out. The slides were examined under a Leitz ortholux microscope fitted with an HBO 200 mercury vapour light source and a 'Ploem' epi-illuminator.

Table 1 lists the mammals examined. Many species were found to exhibit differential binding of the dye to different chromosome areas, giving rise to specific banding patterns. Localized areas showing the much brighter fluorescence of the kind found on the long arms of the human Y chromosome were found in only three species—man, chimpanzee and gorilla. We will refer to this phenomenon as "intense fluorescence".

In man, intense fluorescence was found on the satellites and short arms of some acrocentric chromosomes, the centromere region of chromosome No. 3 and the distal end of the Y chromosome (Fig. 1). The number of autosomes showing

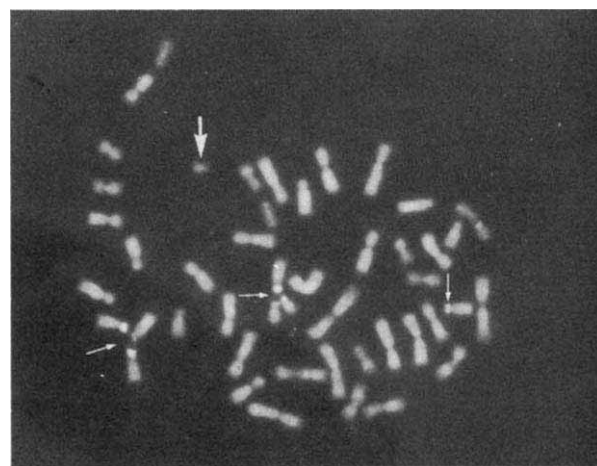


Fig. 2 Chimpanzee metaphase with intensely fluorescent autosomes (small arrows) and a Y chromosome with no intense fluorescence (large arrow).

intense fluorescence and the extent of fluorescence appeared to vary between individuals, some persons showing no intensely fluorescent chromatin and others up to a maximum of seven such discrete areas. From the numbers of acrocentric chromosomes involved and from the fluorescent patterns of the No. 3 centromeres, it was obvious that homologous chromosomes often differ in respect of their patterns of intense fluorescence. The only chromosome consistently found to fluoresce intensely was the Y but even here the length of fluorescing chromatin appeared to vary in direct proportion to variations in the length of the chromosome⁶. The variability between different persons limits the usefulness of the areas of intense fluorescence for systematic chromosome identification, although the banding patterns do seem to have a more consistent appearance and promise to be of much greater value for identifying the chromosomes in the human karyotype⁷.

In the chimpanzee, intense fluorescence was seen on the satellites and short arms of some acrocentric chromosomes, but not on the Y chromosome. With the gorilla, there was also intense fluorescence on acrocentric autosomes and on the centromere region of a pair of large submetacentric autosomes. The distal parts of the long arms of the Y chromosomes were also intensely fluorescent, looking very similar to that of the human Y chromosome (Fig. 3).

Although banding patterns due to the subtle distributions of the fluorochrome at a lower intensity were seen in the chromosomes of all animals examined, they appeared to be

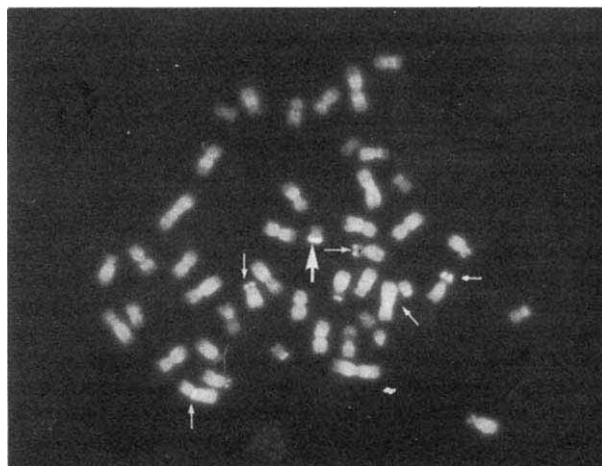


Fig. 3 Gorilla metaphase with intensely fluorescent autosomes (small arrows) and an intensely fluorescent Y chromosome (large arrow).

Table 1 Species Examined for Quinacrine Fluorescence

Species	Sex	No. studied	Intense auto-somal fluorescence	Intense Y fluorescence
<i>Homo sapiens</i>	Man			
	Male	> 100	+	+
	Female	> 100	+	—
<i>Pan troglodytes</i>	Chimpanzee			
	Male	5	+	—
	Female	2	+	—
<i>Gorilla gorilla</i>	Lowland gorilla			
	Male	1	+	+
<i>Pongo pygmaeus</i>	Orang utan			
	Male	1	—	—
	Female	2	—	—
<i>Hylobates lar</i>	Lar gibbon			
	Male	1	—	—
<i>Papio papio</i>	Guinea baboon			
	Male	1	—	—
<i>Papio hamadryas</i>	Sacred baboon			
	Male	1	—	—
<i>Papio cynocephalus</i>	Yellow baboon			
	Male	1	—	—
	Female	2	—	—
<i>Cercopithecus patas</i>	Patas monkey			
	Male	1	—	—
<i>Macaca mulatta</i>	Rhesus monkey			
	Male	2	—	—
	Female	1	—	—
<i>Aotus species</i>	Aotus monkey			
	Male	1	—	—
<i>Cebus capuchinus</i>	Capuchin monkey			
	Male	1	—	—
<i>Bos taurus</i>	Domestic cow			
	Male	2	—	—
<i>Camelus bactrianus</i>	Bactrian camel			
	Male	1	—	—
<i>Sus scrofa</i>	Domestic pig			
	Male	2	—	—
<i>Equus caballus</i>	Horse			
	Male	1	—	—
<i>Equus asinus</i>	Donkey			
	Male	1	—	—
<i>Ovis musimon</i>	Mouflon			
	Male	1	—	—
<i>Felis catus</i>	Domestic cat			
	Male	2	—	—
<i>Panthera tigris</i>	Tiger			
	Male	1	—	—
<i>Lynx canadensis</i>	Lynx			
	Male	1	—	—
<i>Lagostomus trichodactylus</i>	Plains vishcaca			
	Male	1	—	—
<i>Oryctolagus cuniculus</i>	Rabbit			
	Male	1	—	—
<i>Rattus rattus</i>	Rat			
	Male	1	—	—
<i>Mus musculus</i>	Mouse			
	Male	1	—	—
<i>Acomys cahirinus</i>	Spiny mouse			
	Male	1	—	—
<i>Petrogales bennettii</i>	Bennett's wallaby			
	Male	1	—	—

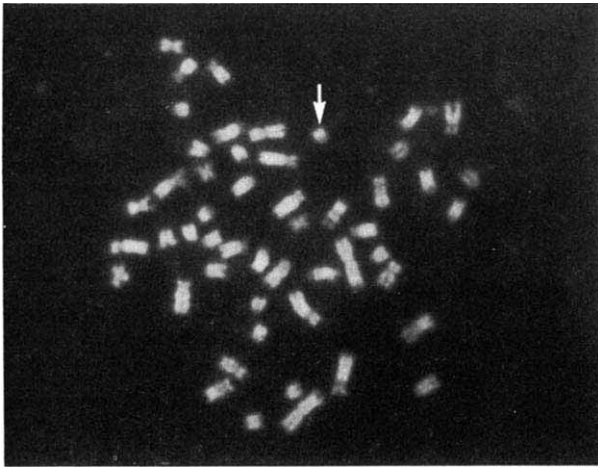


Fig. 4 Aotus monkey metaphase with no intense fluorescence. Y chromosome indicated (large arrow).

enhanced in the primates. Fig. 4 shows the chromosomes from a male aotus monkey with an absence of intensely fluorescent regions but with obvious banding patterns. On the basis of banding patterns, the largest pair of autosomes of most primates studied had a fluorochrome distribution similar to that found in the No. 1 chromosome of man. Other similarities and dissimilarities were noted between species suggesting that further studies with this technique will provide useful information about chromosome homologies.

Fig. 5 shows the fluorochrome distribution given by quinacrine dihydrochloride and ethidium bromide on the chromosomes of Bennett's wallaby. The two dyes give opposite staining distributions, an effect which could not be convincingly demonstrated on the chromosomes of other mammals. The chromosomes of the wallaby, however, are very large and bind large quantities of fluorochrome. As a result, subtle differences in the distribution of fluorochromes can more easily be demonstrated. It is interesting to note that the only two chromosomes in this mammal which did not show a differential affinity for either fluorochrome were the X and Y chromosomes.

So far, intensely fluorescent chromatin has only been found in man, chimpanzee and gorilla, which suggests that possession of such chromatin is probably confined to a closely related group of hominoid apes. Other studies have confirmed the presence of intense fluorescence in certain plant species and in *Drosophila melanogaster* but this must be considered to be independent of the occurrence noted here^{8,9,15}. Our observations confirm previous deductions based on biochemical¹⁰ and cytogenetic^{11,12} data that man and the African great apes have more similarities to each other than to any other primate, including the orang utan which has traditionally been placed in the same sub-family as the great apes. A recent review¹³ of fossil evidence linking man with the great apes suggests that the Hominidae and Pongidae diverged from a Dryopithecine lineage in the early Miocene epoch or earlier. On this basis, it seems that the intense fluorescence present on the chromosomes of the great apes and man has arisen in a common stock at least 20,000,000 yr ago. The absence of intense fluorescence on the chimpanzee Y chromosome does not imply that the gorilla is more closely related to man than the chimpanzee. Indeed, other karyotypic features suggest the opposite. It has been established that variations in the length of the human Y chromosome determine variations in the length of intense fluorescence on the chromosome without causing any known associated phenotypic variations (ref. 6 and personal communication from D. S. Borgaonkar and D. H. Hollander). Similarly, variations in the extent of intense human autosomal fluorescence occur without any known phenotypic variation. These points, taken in conjunction with the observation that fluorescent Y chromatin is tightly condensed at interphase¹⁴, and thus not

involved in gene transcription, make it likely that intensely fluorescent chromatin is genetically redundant. The absence of intense fluorescence on the chimpanzee Y chromosome may therefore be explicable in terms of its small size relative to that of the human and gorilla Y chromosomes in that it contains no such genetically redundant chromatin. Whether evolution has progressed towards deletion of the intensely fluorescent chromatin from the chimpanzee Y chromosome or towards its addition onto the human and gorilla Y chromosomes cannot be assessed from the evidence reported here.

The large variations found in the frequency of intensely fluorescent autosomal areas in man are not reflected, as far as we can see, in the chimpanzee, where there seems to be a more uniform frequency; all specimens from this species which we studied had either six or seven intensely fluorescent satellites or short arms on acrocentric chromosomes. None of the chimpanzees studied were, as far as we know, immediately related to each other, and so this implies that the frequency of intensely fluorescent autosomes has become stabilized at a much higher level than in man.

As regards the other hominoid apes, the absence of intense fluorescence in the orang utan or gibbons makes it likely that they evolved from an ancestral stock before the origin of the lineage giving rise to the African great apes and man.

The presence of banding patterns is related to a physico-chemical differentiation along the chromosomes, which is not well understood. It does not seem to be related to the state of contraction of the chromatin or to follow the normally accepted physical and chemical features of heterochromatin¹⁵. The same observations apply to the intensely fluorescent chromatin. Thus, the inactivated X chromosome in human female interphase nuclei does not fluoresce intensely, but the Y chromatin in male interphase nuclei does. Areas of intense autosomal fluorescence are also occasionally visible in interphase nuclei.

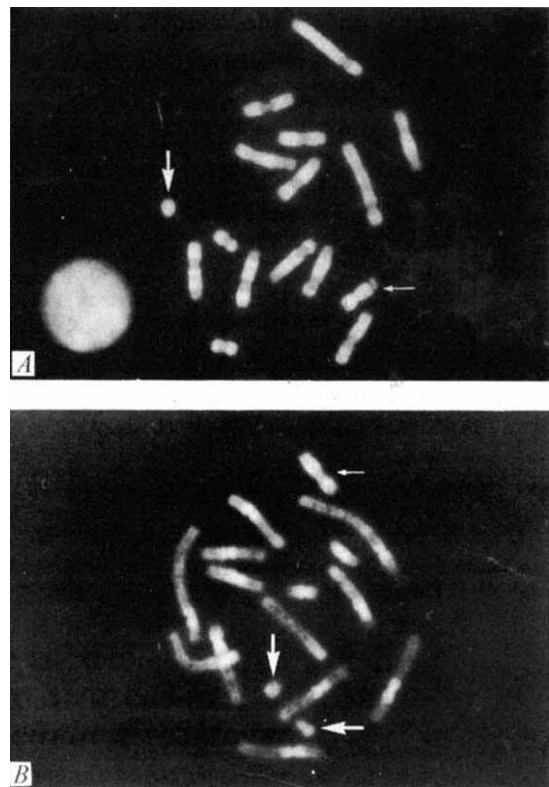


Fig. 5 Metaphase plates of Bennett's wallaby. A, Quinacrine dihydrochloride staining—dull areas principally around the centromeres of the autosomes. The X (small arrow) and Y chromosomes (large arrow) are indicated. B, Ethidium bromide staining—bright areas around the centromeres of the autosomes. This cell contains one X (small arrow) and two Y chromosomes (large arrows).

Our observations indicate that in mammals the sexing of cells, including spermatozoa, by the quinacrine technique only seems feasible on man and, in theory, the gorilla. Lack of material other than lymphocytes has prevented us from verifying this latter point.

The patterns of quinacrine fluorescence have already been useful in tracing obvious similarities and dissimilarities between the chromosomes of some of the species studied. Further work is likely to establish more information on the evolutionary links provided by chromosome morphology.

We thank the following organizations and people for material: Primate Centre, TNO, Rijswijk, Holland; ARC Unit of Reproduction and Physiology, Cambridge; Zoological Society of London; R. Andrews and J. Aspinall, Howletts, Bekesbourne, Kent; Zoological Gardens, Basel; Zoological Gardens, Bristol; Physiology Department, Oxford; London School of Hygiene and Tropical Medicine. P. W. B. acknowledges receipt of a research grant from the Medical Research Council. We also thank Dr C. E. Ford for comments on the manuscript.

P. L. PEARSON
M. BOBROW

*MRC Population Genetics Unit,
Old Road, Oxford*

C. G. VOSA

*Botany School,
University of Oxford*

P. W. BARLOW*

*Paediatric Research Unit,
Guy's Hospital, London*

Received November 24, 1970.

* Present address: Zoology School, University of Oxford.

- ¹ Caspersson, T., Farber, S., Foley, G. E., Kudynowski, J., Modest, E. J., Simonsson, E., Wagh, U., and Zech, L., *Exp. Cell Res.*, **49**, 219 (1968).
- ² Caspersson, T., Zech, L., and Johansson, C., *Exp. Cell Res.*, **60**, 315 (1970).
- ³ Zech, L., *Exp. Cell Res.*, **58**, 463 (1969).
- ⁴ Pearson, P. L., and Bobrow, M., *J. Reprod. Fert.*, **22**, 177 (1970).
- ⁵ Arakaki, D. T., and Sparkes, R. S., *Cytogenetics*, **2**, 57 (1963).
- ⁶ Bobrow, M., Pearson, P. L., Pike, M. C., and El-Alfi, O. S., *Cytogenetics* (in the press).
- ⁷ Caspersson, T., Zech, L., Johansson, C., and Modest, E. J., *Chromosoma*, **30**, 215 (1970).
- ⁸ Caspersson, T., Zech, L., Modest, E. J., Foley, G. E., Wagh, U., and Simonsson, E., *Exp. Cell Res.*, **58**, 128 (1969).
- ⁹ Vosa, C. G., *Chromosoma*, **31**, 446 (1970).
- ¹⁰ Goodman, M., *Classification and Human Evolution* (edit. by Washburn, S. L.), 204 (Aldine, Chicago, 1963).
- ¹¹ Bender, M. A., and Chu, E. H. Y., *Evolution and Genetic Biology of Primates* (edit. by Buettner-Janush, J.), 261 (Academic Press, New York, 1963).
- ¹² Hamerton, J. L., Klinger, H. P., Mutton, D. E., and Lang, E. M., *Cytogenetics*, **2**, 240 (1963).
- ¹³ Buettner-Janush, J., *Origins of Man* (Wiley, New York, 1966).
- ¹⁴ Pearson, P. L., Bobrow, M., and Vosa, C. G., *Nature*, **226**, 78 (1970).
- ¹⁵ Vosa, C. G., *Chromosoma*, **30**, 366 (1970).

Absence of Cell Sorting Out in the Grex of the Slime Mould *Dictyostelium discoideum*

THE development of the cellular slime moulds demonstrates very simple pattern formation. Cells from an apparently homogeneous population come together to form an aggregate which differentiates into a pattern of only two types of cell, stalk and spore. These are rearranged spatially to form the fruiting body. A striking feature of this pattern is that the proportions of stalk and spore are relatively constant over a wide range of size of aggregate¹. Although the aggregate can

differentiate directly into a fruiting body² it usually migrates as a coordinated irritable whole in the form of a tapered cylinder called the grex. It is known that any sufficiently large part of the migrating grex can give rise to a normal fruiting body, and in this sense the development of the slime mould shows considerable regulation^{3,4}. At present most workers accept that during migration the front third of the grex becomes prestalk cell and rear two-thirds prespore¹. It is necessary for an understanding of pattern formation to know when this pattern is specified and when the cells become determined. In view of the capacity of the grex to regulate, it is of great interest that histochemical and ultrastructural differences between prespore and prestalk cells in the grex have been found by various workers^{1,5-9}, because such differences are clearly reversible.

One way in which the pattern could be specified would be by the establishment of a gradient along the axis of the grex which could effectively provide the cells with positional information¹⁰. If the cells have their position specified with respect to the ends of the grex it is not too difficult to envisage mechanisms whereby the front third become stalk and the rear two-thirds spore. Takeuchi⁷ has claimed that several cell properties are randomly distributed among the pre-aggregation population of amoebae, and that during aggregation they sort out into detectable gradients in the grex. This raises the question as to whether the properties of the cells can be changed by changing their position in the gradient. In this connexion Bonner has claimed that cells "know" their position within the grex^{5,6}. He reported that cells returned to their original position in the grex after being grafted to either front or rear. It is surprising that the cells have a well determined property which enables them to return to their original position. One would have expected, on the basis of positional information, that they would take on the properties of the region to which they were transplanted. Because of the importance of this point, we have attempted to confirm Bonner's observation.

Bonner^{5,6}, following the work of Raper³, found that the cells could be labelled with vital dyes such as Nile blue and cresyl violet. He grafted the heads of grexes stained with Nile blue onto the rear end of unstained grexes and reported that blue cells moved up the grex over a period of 6 h to assume an anterior position, implying that the cells he had introduced "knew" they were anterior cells and moved to their correct position. He also claimed the same result in the complementary experiment in which labelled cells from the tail of one grex moved down the body of another when grafted into its tip. Our preliminary investigations revealed that all the water soluble vital dyes used, including Nile blue, neutral red, bismark brown and cresyl violet, and fluorescent ones like RB200 and fluorescein, were transferred from cell to cell, so that a mixture of equal numbers of labelled and unlabelled cells gave, within a few hours, a population of uniformly labelled cells. These observations (together with the inherent ignorance of the processes actually involved) cast serious doubt on the suitability of vital dyes as cell markers. A new label was obtained by following a suggestion of Gerisch. Acridine orange was adsorbed onto microgranular diethyl-amino-ethyl cellulose, and the particles then fed to the myxamoebae. Whatman 'CM32' ion exchange resin was sonicated in a 2% solution of acridine orange to obtain small particles and fragments below 2 μ m in diameter were collected by differential centrifugation. These were soaked in the 2% acridine orange solution for 24 h and then all the unadsorbed dye was removed by repeated centrifugal washing in distilled water.

D. discoideum was grown at 22° C in association with *E. coli* B/r on Sussman's standard medium¹. Forty-five hour old cultures were collected in ice cold Bonner's solution and washed twice by centrifugation at 180g for 4 min to remove the bacteria. The myxamoebae from each plate (about 2×10^7 cells) were made up to 2 ml. with either Bonner's solution or

resin suspension containing 10^8 particles/ml. The cells were then dispensed onto the surface of non-nutrient agar plates, at approximately 0.6 ml. per plate. These were incubated at 22° C in the dark, in an atmosphere saturated with water vapour, for 12 h, after which migrating grexes had formed. Observed under ultraviolet light, of incident wavelength 3,300 Å to 4,600 Å, resin-treated cells autofluoresced yellow-green, the resin showing up as bright orange particles within each cell.

Labelled cells behaved normally, the label not affecting development. Further, there was little transfer of label from cell to cell: mixture of equal numbers of labelled and unlabelled cells showed $50\% \pm 2\%$ of the cells labelled after 2.5 h in suspension or 8 h on an agar surface.

At the beginning of migration 85–95% of grex cells were labelled. There was a progressive loss of label from the rear end of the grex during migration, so that after 12 h there were 85% labelled cells at the head and 70% at the tail. In some newly formed grexes the label was not randomly distributed, but was arranged in several discrete transverse bands, the origin and significance of which are not understood. During the gross morphological changes of culmination most of the resin was egested by the cells as they dehydrated to become rigid, dead, stalk cells with heavy cellulose walls, or highly condensed, refractile spores. The label therefore seemed satisfactory for studies of cellular distribution in the slime mould, up to the time of culmination.

Generally, grexes which had been migrating for 2–3 h were used. Grafting was done in a 'Perspex' cabinet maintained at 18–20° C and at a relative humidity greater than 85%. Suitable grexes were transferred to new agar plates for grafting, then the plates were kept at 22° C and 100% relative humidity for 4–6 h. Grafts were examined after the longest possible migratory period. For examination the grexes were either squashed under a coverslip, or cut into thirds, so that the percentage of labelled cells in each piece could be counted. Examinations were done on a Zeiss Universal Microscope ($\times 800$ or 1,250) under fluorescent illumination (exciter filters BG12, BG38, barrier filters 41 and 50).

Preliminary grafts showed no major intrusion of labelled region into unlabelled, in all cases the zone of mixing being less than 50 μ m (six or seven cell diameters). Grafts of labelled heads into the rear of unlabelled grexes showed all the label in the rear after up to 5 h migration, the junction being a mixture of labelled and unlabelled cells only two or three cells wide. Grafts of labelled tails onto the fronts of unlabelled grexes (though difficult to accomplish successfully) again show no gross cell sorting: the labelled cells moving no further than the junction zone, in these cases about six or seven cells wide. Similar results were obtained grafting unlabelled heads and tails onto labelled grexes. All such experiments showed that cells remained where they were placed in the grex (irrespective of the age of either host or donor grex). Our experiments show that less than 4%, if any, of the cells return to their original positions.

It is very difficult to reconcile these findings with those of Bonner, though it is certain that our labelling technique is more reliable than those involving vital stains. We therefore believe that there is no cell sorting out in the migrating grex of the slime mould, and no irreversible positional determination. It is possible that there are differences in cell type in the grex, but it may well be that these differences merely reflect axial gradients of cell properties involving, for example, migration or slime sheath production, and may have nothing to do with the development of pattern. Even if such a pre-pattern does arise in the migrating grex it is obvious that it is not essential for the construction of a normal fruiting body; pieces of grex regulate and fruit normally³ and cell aggregates can be made to form fruiting bodies without ever going through a migrating grex phase². This suggests that the essential processes specifying pattern might take place just before or even during culmination and is supported by our preliminary investigations

of this stage. These show both that cells are not determined until they show morphological changes to spore and stalk, and that it is still possible to produce multiple fruiting bodies even after culmination has begun.

We thank Dr D. Garrod for constructive criticisms. This work was supported by the Science Research Council.

P. A. FARNSWORTH
L. WOLPERT

Department of Biology as Applied to Medicine,
The Middlesex Hospital Medical School,
London W1

Received July 24, 1970; revised March 18, 1971.

- ¹ Bonner, J. T., in *The Cellular Slime Moulds* (Princeton University Press, 1967).
- ² Gerisch, G., *Current Topics Develop. Biol.*, **3**, 157 (1968).
- ³ Raper, K. B., *J. Elisha Mitchell Sci.*, **56**, 241 (1940).
- ⁴ Raper, K. B., *Growth Supp. (Symp. 3)*, **5**, 41 (1941).
- ⁵ Bonner, J. T., *Amer. Naturalist*, **86**, 78 (1952).
- ⁶ Bonner, J. T., *Quart. Rev. Biol.*, **32**, 232 (1957).
- ⁷ Takeuchi, I., in *Nucleic Acid Metabolism, Cell Differentiation and Cancer Growth* (edit. by Cowdry and Seno) (Pergamon Press, 1969).
- ⁸ Takeuchi, I., and Sato, T., *Jap. J. Exp. Morphol.*, **19**, 67 (1963).
- ⁹ Hohl, H. R., and Hamamoto, Susan, *J. Ultrastruct. Res.*, **26**, 441 (1969).
- ¹⁰ Wolpert, L., *J. Theoret. Biol.*, **25**, 1 (1969).

Rediscovery of the Marsupial *Echymipera rufescens* in Australia

THE marsupial faunas of both New Guinea and Australia indicate that there has been a continuing interchange between the two land masses with Cape York Peninsula as the main gateway into and out of Australia. Of the bandicoots (family Peramelidae) only two species occur in both Australia and New Guinea. The short-nosed bandicoot *Isodon macrourus* Gould, common in eastern Australia and Arnhem Land, is found also in southern and south-eastern Papua and possibly migrated from Australia to this region¹. The spiny haired bandicoot, *Echymipera rufescens* Peters and Doria, is the only terrestrial marsupial found in the rainforests of Cape York Peninsula which is considered to be of New Guinean origin². This bandicoot is widespread in the forested foothills and lowlands of western New Guinea both north and south of the central mountain range as well as south-western Papua and the Kei and Aru islands³. In Australia it is known only by a single specimen caught in 1932 by P. J. Darlington. This animal was described by Tate¹ and given the status of a new subspecies *Echymipera rufescens australis*. In this paper¹ the type locality was given as "Rocky Scrub", Rocky River, but later Tate² gave it as the upper Nesbit River. In spite of extensive efforts by the 1948 Archbold Expedition and later groups, no more *E. r. australis* were caught and the subspecies was considered to be extremely rare⁴.

In August 1970, during an expedition to the Cape York Peninsula, we managed to capture five *E. rufescens*, two adults (a male and a female) and three sub-adults (two females and a male). They were live trapped using a peanut butter-oil-oats mixture as bait. Trapping effort comprised approximately 300 trap nights, running traps for 2 to 3 nights in a region before shifting them elsewhere. An adult (male) and sub-adult (female) were caught on the Rocky River on the eastern slopes of the McIlwraith Range (18 miles ENE of Coen), the other animals being caught on the McIlwraith Range near the head of Attack Creek (30 miles NNE of Coen), a tributary of the Archer River. All the animals were caught in tropical rainforest and near flowing water. At Attack Creek the rainforest formed a large continuous tract with a canopy 15–20 m tall in the trapping area. Ground cover consisted of sparse low and medium height shrubbery so that the forest floor was

relatively open. Numerous small conical diggings, similar to those of other bandicoots, were seen on the sandy soil floor of the rainforest gullies, especially nearer to creeks. At the Rocky River site, the rainforest was present in the form of a gallery forest on the river and adjoining gullies. The tract containing the bandicoots was tall (30–40 m) and open at the lower level with little development of shrub layers.

The bandicoots of the genus *Echymipera* are distinguished from other Australian bandicoots by four pairs of upper incisors as distinct from the five pairs in all other Australian species. The animals captured had dark brown heads with long pointed muzzles. The rump was rufescent and the tail short, black and naked. The belly fur was almost cream in colour. The fur was very spiny with virtually no soft underfur and thus probably offers little insulative protection. This may be an adaptation to the tropical environment. Both adults weighed 800–850 g when caught and the smaller animals were of a similar size, 300–450 g. The skull measurements of one of the adults which died in captivity were not markedly different from the measurements made by Tate¹ on the only other Australian specimen and therefore similar to the skull characteristics of the New Guinea animals. Further studies, perhaps using biochemical markers, will therefore be necessary to confirm the status of this Australian sub-species.

The presence of *E. rufescens* in Australia is now confirmed and its known range in this country extended. Although the range is not large the species appears to be reasonably common within this limited area.

This work was supported in part by a CSIRO postgraduate studentship grant and in part by a grant from the Australian Research Grants Committee.

A. J. HULBERT
G. GORDON
T. J. DAWSON

School of Zoology,
University of New South Wales,
Kensington,
New South Wales

Received March 11, 1971.

¹ Tate, G. H. H., *Bull. Amer. Mus. Nat. Hist.*, **92**, 313 (1948).

² Tate, G. H. H., *Bull. Amer. Mus. Nat. Hist.*, **98**, 563 (1952).

³ Laurie, E. M. O., and Hill, J. E., *List of Land Mammals of New Guinea, Celebes and Adjacent Islands, 1758–1952* (British Museum (Nat. Hist.), London, 1954).

⁴ Ride, W. D. L., *A Guide to the Native Mammals of Australia* (Oxford University Press, Melbourne, 1970).

Antitranspirant Activity of the Methyl and Phenyl Esters of Absciscic Acid

CERTAIN analogues and derivatives of abscisic acid (ABA) have growth inhibitory effects similar to those of the parent compound^{1–3}. Although most are less effective than ABA itself, Koshimizu *et al.*³ found that some esters were more effective, and suggested that they could penetrate into the plant more easily than the free acid. We have now found that two esters can cause stomatal closure and reduce transpiration in doses which do not inhibit growth appreciably.

The methyl and phenyl esters of ABA were synthesized for us by Shell Research Ltd. Stomatal apertures were measured by means of an automated resistance porometer⁴ on plants in growth cabinets kept at 20° C with light of 8,000 lux. The air supply to the porometer cups had a constant water vapour deficit of 11.6 mbar, and a carbon dioxide concentration of either zero or 330 p.p.m. (ambient air). Measurements of

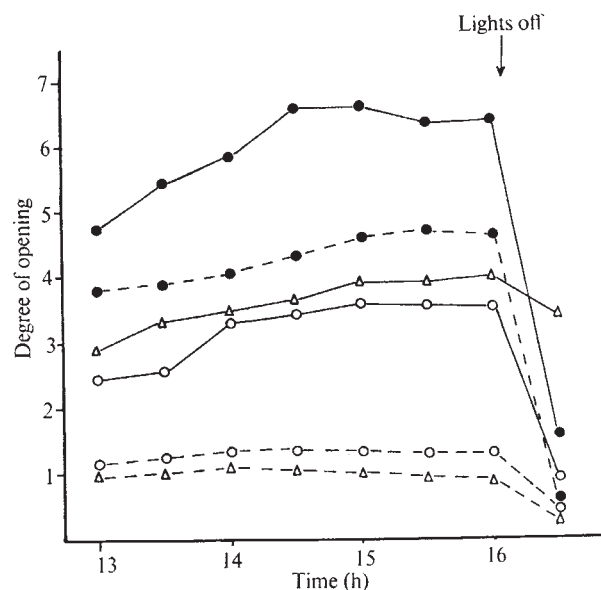


Fig. 1 ○, Stomatal behaviour of leaves of *Xanthium strumarium* treated with the phenyl ester of ABA; △, treated with the methyl ester. ●, The observations on untreated control plants. —, Treatment with CO₂-free air; - - -, 330 p.p.m. CO₂ (ambient air). Stomatal opening is in the arbitrary units of the recording porometer. Each point is the mean of three observations. The treatments were significantly different from the controls (P 0.01–0.001).

total transpiration were made on whole plants grown in water culture.

Both the methyl and phenyl esters, applied once to the leaf surface giving a total dose of approximately $0.1 \mu\text{g cm}^{-2}$, caused a highly significant suppression of stomatal opening in *Xanthium strumarium* (Fig. 1). The effect was clearly of similar magnitude in both normal and CO₂-free air, and in other experiments we found that the CO₂ compensation point was unaffected. This suggests that the compounds act directly on the stomatal apparatus and do not induce closure simply by increasing the internal CO₂ concentration. These results are similar to those obtained with ABA itself⁵ and suggest that these two derivatives have a similar mode of action to the naturally occurring compound.

Transpiration trials were carried out with young plants of barley (variety 'Sultan'), which had received one surface application of the esters at the three or four leaf stage. There was a marked reduction in transpiration which was still apparent after 9 days (Fig. 2), and which was not accompanied by any

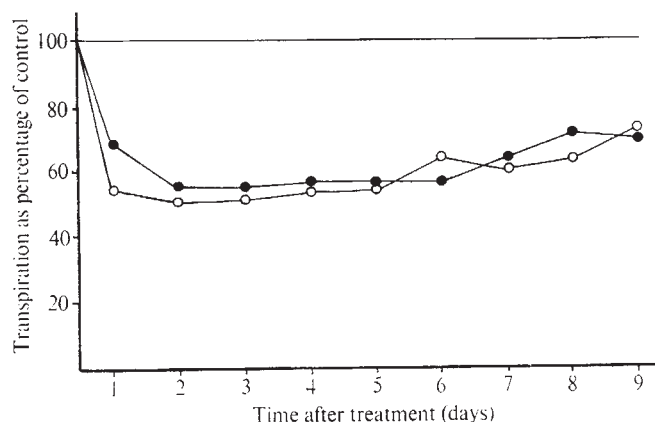


Fig. 2 Transpiration of whole plants expressed as a percentage of the controls. ●, Methyl ester; ○, phenyl ester; —, control. Each point is the mean of five observations. Transpiration after treatment with the esters was significantly less than the control level ($P < 0.001$).

significant slowing of growth. In fact, the transpiration ratio (the ratio of total transpiration (in g) to total dry matter production (in g) during a given interval of time) decreased in the period of the experiment. This ratio indicates the degree of inhibition of growth by an applied substance⁶. The results reaffirm that the primary effect of the esters is on the stomata, and that the efficiency of mesophyll photosynthesis is not appreciably altered. No other toxic effects on the plants have been found.

The suppression of stomatal opening is slightly more persistent with the esters than with ABA. This may be a result of greater penetration into the plant, or of breakdown over a period of time. A compound which releases ABA by slow hydrolysis might be more effective than the same dose of the active acid applied at one time, which could then be subject to rapid inactivation⁷.

The methyl and phenyl esters of abscisic acid thus seem to function effectively as antitranspirants, and because of their prolonged action may be suitable for protecting field crops during droughts.

We thank the Countess Eleanor Peel Trust for financial support, and Mr T. Chapman of Shell Research Limited for supplies of the two esters.

RUTH J. JONES
T. A. MANSFIELD

Department of Biological Sciences,
University of Lancaster

Received February 26, 1971.

- ¹ Tamura, S., and Nagao, M., *Planta*, **85**, 209 (1969).
- ² Sondheimer, E., and Walton, D. C., *Plant Physiol.*, **45**, 244 (1970).
- ³ Koshimizu, K., Fukui, H., and Mitsui, T., *Agric. Biol. Chem.*, **30**, 941 (1966).
- ⁴ Allaway, W. G., and Mansfield, T. A., *Canad. J. Bot.*, **47**, 1995 (1969).
- ⁵ Jones, R. J., and Mansfield, T. A., *J. Exp. Bot.*, **68**, 714 (1970).
- ⁶ Slatyer, R. O., and Bierhuizen, J. F., *Agric. Meteorol.*, **1**, 42 (1964).
- ⁷ Milborrow, B. V., *Sci. Prog.*, **57**, 533 (1969).

Auxin Production by Phylloplane Fungi

DURING a study of the distribution of fungi on living leaves of sycamore (*Acer pseudoplatanus*) a succession of filamentous forms has been found, with *Aureobasidium pullulans* colonizing the expanding leaves, followed by *Cladosporium herbarum* and finally by *Epicoccum nigrum*. These three species have commonly been found on the surfaces of green leaves of a wide range of plants which have been studied by many workers. The veinal distribution of these fungi on sycamore led to a search for them in surface-sterilized leaves, and all three species have been isolated¹. *Aureobasidium*, however, has also been isolated from surface sterilized bud scales, shoots and even from the roots of seedlings, and it has been described as an endophyte in sycamore and other trees². The relationship between *Aureobasidium* and the higher plant could be in the form of symptomless parasitism, or, conversely, there could be some benefit to, or other influence on, the higher plant. Aspects of the physiology of the three common filamentous phylloplane fungi have been investigated to see if growth promoting substances can be produced by them, for Jump³ suggested that a phytohormone produced by *A. pullulans* (*Dematiu pullulans*) may have been responsible for forking in red pine.

The three fungi were grown at different temperatures and for various times on a reciprocal shaker, with 100 ml. of Czapek's Dox medium in 1 l. flasks to ensure adequate aeration. At the end of the growing period the medium was checked to ensure that there was no contamination. The mycelium was separated by centrifugation, and the culture filtrate was used in a dilution series at 25° C with *Avena* coleoptiles for evidence of growth-promoting substances. When tryptophane was used as the N

source there was a marked effect of the culture filtrates on the coleoptiles compared with controls which were incubated in distilled water or in uninoculated culture medium. Maximum elongation was obtained with filtrates from cultures of *Aureobasidium* and *Epicoccum* which had been grown at 15° C rather than at 5° C or at 25° C. Cultures of *Aureobasidium* which were collected after 7 days caused a 62% elongation of the coleoptiles compared with the controls. *Epicoccum* cultures harvested after 15 days of incubation produced an elongation of 55% over the controls. *Cladosporium* was incubated at 5° C and at 25° C, and the filtrates caused elongations of 41% and 35% after 21 days and 7 days of incubation respectively.

Aureobasidium and *Epicoccum* were subsequently grown for 7 days at 25° C in diffuse light on a reciprocal shaker, and the mycelium separated by centrifugation. The filtrate was extracted at 2° C for 16 h with peroxide-free ether. The extract was evaporated to dryness in a rotary evaporator at 30° C, and taken up into a small volume of ether for chromatography, when isopropanol/NH₃ (specific gravity 0.880)/H₂O (10:1:1 v/v/v) was found to be the most satisfactory solvent. The three sprays used were Ehrlich's and Salkowski's reagents, and Prochazka's reagent was used for fluorescence in ultraviolet light.

The chromatograms of the total ethereal extract from the *Aureobasidium* culture filtrate showed five well-defined spots, but three of the five spots from the *Epicoccum* extract were not as clearly separated. One of the five spots from both fungi corresponded closely in colour reaction and *R_F* value to indole-3-acetic acid (IAA), and elution of unsprayed chromatograms indicated 0.15 µg/ml. and 0.05 µg/ml. of IAA for *Aureobasidium* and *Epicoccum* respectively. A second spot from *Aureobasidium* and the three poorly separated spots from *Epicoccum* overlapped the indole-3-aceto nitrile (IAN) spot. The *R_F* values were similar, and colour reactions approximated to those for IAN. Elution of unsprayed chromatograms indicated 0.065 µg/ml. and 0.0125 µg/ml. from *Aureobasidium* and *Epicoccum* respectively. Valadon and Lodge⁴ have shown that both IAA and IAN can be produced by *Cladosporium* at 0.015 µg/ml. and 0.02 µg/ml. respectively.

The production of these and possibly other auxins *in vitro* is of potential interest to all who work with leaves, and to those who work on factors which influence bud and seed dormancy. Tukey⁵ has shown that auxins and their possible precursors can be found in plant leachates, and that leachates can be absorbed by both leaves and roots. Some, at least, of these auxins may have been produced by fungi living on leaf surfaces or even within the tissues of the higher plants. The production of auxins by phylloplane fungi *in vivo* would suggest that these fungi may play a much fuller role in nature, for example in growth regulation within the ecosystem, than has previously been suspected. Further studies on these interrelationships are being carried out.

N. G. B. acknowledges the award of a research studentship by the Scientific Research Council.

N. G. BUCKLEY*
G. J. F. PUGH

Department of Botany,
University of Nottingham,
University Park,
Nottingham NG7 2RD

Received February 24; revised March 16, 1971.

* Present address: Plant Protection Ltd, Jealotts Hill Research Laboratories, Bracknell, Berkshire.

- ¹ Pugh, G. J. F., and Buckley, N. G., in *The Ecology of the Leaf Surface* (Academic Press, London and New York, in the press).
- ² Pugh, G. J. F., and Buckley, N. G., *Trans. Brit. Mycol. Soc.* (in the press).
- ³ Jump, J. A., *Phytopathology*, **28**, 798 (1938).
- ⁴ Valadon, L. R. G., and Lodge, E., *Trans. Brit. Mycol. Soc.*, **55**, 9 (1970).
- ⁵ Tukey, jun., H. B., in *The Ecology of the Leaf Surface* (Academic Press, London and New York, in the press).

BOOK REVIEWS

Appalachians Discovered

The Tectonics of the Appalachians. By John Rodgers. (Regional Geology Series.) Pp. xii+271. (Wiley Interscience: New York and London, February 1971.) £11.50.

FROM time to time a book appears which treats its subject so successfully as to illuminate a larger problem. A book of this description can interest a wider readership than its title might at first sight suggest. John Rodgers has written just such a book, for his account of the tectonics of the Appalachians not only provides a first class description of the geological history of that mountain chain but indirectly throws light on a more general issue—the question as to how the present day morphology of the Earth is related to older structures dating from Palaeozoic and Mesozoic times and how these in their turn may have been influenced by still older Precambrian events. For several reasons—location, age, internal make-up and the care with which it has been investigated—the Appalachian chain is of critical importance in this connexion.

The chain was not only once connected, as Rodgers shows, to equally old structures in West Africa and in north-west Europe, but is linked in some way to the remarkable Ouachita mountains of Arkansas and Oklahoma. Moreover, the Appalachians were affected by a variety of Mesozoic and Tertiary events which led in due course to the opening of the Atlantic in its immediate vicinity after mountain building had ended.

Rodgers discusses both the former relationship with mountain chains outside North America and the history of his region after the folding of the Appalachians was complete. The principal justification for a study of the tectonics of the Appalachians must, however, lie in the diversity of structures which the long record of investigation has revealed and which form the main subject of this able synthesis. This is field geology at its best.

It was on May 23, 1540, that de Soto recorded traces of gold as he led the first European explorers up the Savannah River into the southernmost limits of the mountain system which was so decisively to influence the pattern of settlement and government in North America. Some two hundred and fifty years later, the close juxtaposition of the mountain belt and the chain of centres of population and learning—a circumstance matched in Europe only in Switzerland—led to the stream of geological discoveries which have continued to this day. Since 1809, when William Maclure, a settler from Ayrshire, published his synthesis of Appalachian geology illustrated by the first geological map of the United States, the region has become renowned for the clarity with which the relations between folded and undisturbed rocks are displayed. From this situation emerged in the nineteenth century the notion of the geosyncline, an understanding of the importance of compression as a cause of folding in the crust and an appreciation of the facies changes between foreland and fold belt and of the two contrasting faunas found east and west of the mountain chain. In this century we can recall Hess's grasp of the significance of serpentine belts, his understanding of the interplay between sedimentation and tectonics and the deciphering of the record preserved in the crystalline rocks of the Appalachians by such men as Cloos and Billings in the field and by a host of geochronologists. All this is admirably summarized by Rodgers, who is particularly successful in combining factual descriptions with discussions of the underlying concepts which have emerged from successive discoveries.

The main body of the book carries us chapter by chapter through eight provinces arranged, as the author puts it, "in the order in which our basic understanding of their structure has been achieved". Each chapter outlines the Palaeozoic, and, where appropriate, the Precambrian history of a part of the

Appalachians between the Blue Ridge Mountains and Newfoundland. The book finishes with one chapter on the Triassic basins and related rocks, another which sets out an overall view of the geological history of the Appalachians, and a closing chapter entitled "Generalities and speculations", which is a model of its kind.

There are a small number of well-drawn maps and sections within the text, an excellent tectonic map of the Appalachians in two sheets in an end pocket, a good index and a full and up-to-date bibliography. A lot of thought must have gone into this book and the presentation of information is very good indeed. For many years ahead, this should be the source to which one will first turn for information on Appalachian structures.

What of the wider issues mentioned earlier? The book presents a well documented account of the history of 3,000 kilometres of mountain chains over some 600 million years. It shows how the accumulation of wedges of late Precambrian clastics first differentiated the belt from the remainder of America about 800 million years ago, a situation which can be matched in north-west Europe and Greenland. These wedges for the most part overlie Grenville crystalline rocks and so contrast with the Precambrian sediments of western USA which began to form over 1,200 million years ago on a still older basement.

As Rodgers emphasizes, there was repeated deformation and plutonic activity until about 400 million years ago, when the northern Appalachians and the Caledonian continuation became stabilized while the southern Appalachians and Hercynides remained as mobile belts. In Triassic times when the whole Appalachians were stabilized, clastic deposits, rift systems, and a 1,400 kilometre long belt of basic intrusions were formed within the Appalachians. Still later, the Atlantic opened in this vicinity. Well documented accounts of changes such as

these, in tracts as long as the Appalachians, are essential preliminaries to the understanding of the relations of structures on either side of the Atlantic and ultimately to a comprehension of the global tectonics of the past.

Few geological problems have been investigated for 400 years by scientists from so many countries, for nowhere else in the world has the population on one side of an ocean opened by continental drift migrated in such numbers to the opposing shore. It is a fitting tribute to this long collaboration that Rodgers, an American, should dedicate the book to the memory of Fallot and that it appears in a series edited by another great European structural geologist, de Sitter. Probably neither de Soto nor Maclure ever dreamed that the New World they had entered was once linked to the one they had left.

J. SUTTON

Busy as Bees

Insect Pollination of Crops. By John B. Free. Pp. xi + 544. (Academic: London and New York, December 1970.) £7.25.

It is salutary to be reminded that mankind is dependent on the success of pollination for all plant products which are used for food or as processed com-

modities, which is why it seems rather strange that this should be the first comprehensive review of the subject. But, as Dr Free so clearly demonstrates, mechanical transfer of pollen from anther to stigma is only one facet of a complex and fascinating process which, if successful, leads to fertilization of the ovule. Full understanding of these mechanisms requires a study of the behavioural characteristics of insects which must be closely integrated with morphological features and physiological processes of the plant.

This book is a detailed review of the literature, in which botanical aspects receive as much attention as the insects. The first part, although entitled "Insect Pollinators", deals exclusively with species that can be manipulated to a greater or lesser extent by man—honey bees, bumble bees and a few species of wild bees. All the salient information on behaviour, management and utilization is presented clearly and concisely. To satisfy the curiosity of a reader who wishes to know which species other than Hymenoptera are associated with pollination; to discover, for instance, that midges pollinate para rubber, or that humming birds are thought to pollinate pineapple, or even to be disillusioned about the importance of pollen beetles (which, incidentally, do not even get into the index) in relation to Brassicas,

it is necessary to consult the animal index. It then becomes clear that these receive detailed comment in later chapters dealing with their respective host plants. Even at the expense of some duplication, I would have preferred a comprehensive list of all species in the first section.

The remaining two-thirds of the book consist of a chapter on each plant family which produces edible or other useful products, but excludes ornamentals, timber and those plants in which wind plays the dominant role in dispersal of pollen. Here, botanical features receive the same detailed attention as was given earlier to the social Hymenoptera. The morphology of the flower is illustrated, the period when anthers dehisce, when the stigma is receptive, germ tube growth, and composition of the nectar are all described in detail, together with insects associated with pollination and results of experiments on insect pollination. A recurrent feature of experimental work, which has been largely concerned with honey bees, is the lack of consistency between results obtained by different workers, doubtless because of the empirical approach or the insufficiency of data, with the result that the author has been unable to draw more than tentative conclusions about the benefits obtained. However, with intensification of agriculture, the trend towards monoculture and increasing demands for higher yields and greater uniformity of produce, it is clear that, with many crops, optimal yields will be obtained only if wild pollinators are supplemented by species that can be easily manipulated.

To avoid the shortcomings of much earlier experimental work this book, a distillation of current knowledge, should be compulsory reading for all future workers, whether in cool, temperate or tropical regions. It is essentially a reference book, meticulously cross-referenced, with a huge bibliography and a good index.

G. H. L. DICKER

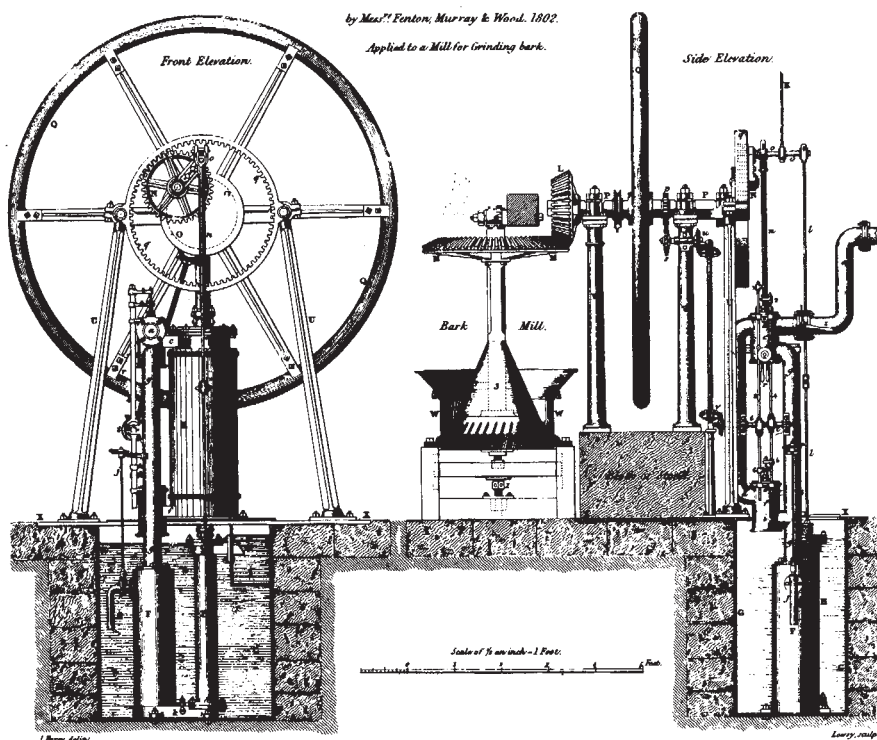
Burning Issues

The Chemistry and Uses of Fire Retardants. By John W. Lyons. (Wiley-Interscience: London and New York, January 1970.) £5.75.

THE ease with which thin sections of wood and fibrous cellulosic materials and textiles catch fire has been known for centuries, and treatments have been sought to reduce this risk. The use of flammable synthetic polymers as substitutes for, and in addition to, cellulosic materials has been increasing rapidly during the second half of this century, and the search for and use of flame retardant treatments has been extended

Nineteenth Century Steam Engine

STEAM ENGINE of 4 Horse Power.
by Mac'F. Fenton, Murray & Wood, 1802.
Applied to a Mill for Grinding bark.



Published as the Act done, by Longman, Rees, Green, Brown & Co., Stationers, 1802.

Plate XVII from John Farey's *A Treatise on the Steam Engine* (1827) now reprinted by David and Charles, £8.40.

to these materials. In this book, which can be regarded as an extension to the excellent monographs on flame retardants for textiles by Ramsbottom (J. E. Ramsbottom, *The Fireproofing of Fabrics*; HMSO: London, 1947) and by Little (Robert W. Little (editor), *Flameproofing Textile Fabrics*; Reinhold: New York, 1947) published in 1947, Dr Lyons has attempted to present the current state of knowledge of flame retardants for all these materials, providing under one cover information from a widely scattered scientific and technical literature.

After a general introductory chapter, the subject matter is presented under two broad classifications: first, flame retardants, and, second, the materials to be treated. This form of presentation leads to some repetition but has the advantage of collecting in each chapter information on a specific agent or flammable material. There is a copious and up-to-date bibliography to assist readers who wish to pursue their enquiries further.

The introductory chapter includes a large section on fire-testing, and smaller sections on the mechanisms of flame retardance, synergistic combinations, and effective concentrations of retardants. The standard methods of test considered are American, but as these are widely adopted and many of the European standard tests are similar in concept, the results obtained can be interpreted for the European markets. A significant omission is a discussion of the relation between tests and conditions of risk, although a brief comparison is made between tests. Many flame retardant treatments have been shown to prevent or delay ignition from a small source, but to have a limited effect in a fire environment beyond an initial delay in the spread of flame. Many of the standard tests for the flammability of materials examine ease of ignition only, and it is unlikely that flame retardant materials will be used in the absence of untreated flammable materials that will burn freely. Thus more needs to be done to discourage the belief that a flame retardant treatment may convert the behaviour of an organic material into that of an incombustible one.

Many of the data in this and subsequent chapters are presented in tabular form. These tables are admirable for the rapid survey of a topic, but some care needs to be exercised in making comparisons between tables. For example, in tables of the properties of materials treated with flame retardants, the tests adopted may not be fully described, the units for specific properties may differ or be incompletely defined and the surface:volume ratios of the materials under test are not always given. The surface:volume

ratio is important, as the concentration of fire retardant needed for a given performance usually increases with this ratio.

The discussion of flame retardancy is not restricted to additives, but includes retardants incorporated into polymeric structures and surface coatings; brief mention is also made of methods of control of forest fires and of reducing the fire risk with liquid fuels. A notable omission is a discussion of the special conditions of exposure in hyperbaric or oxygenated atmospheres, which can drastically reduce the efficiency of fire retardant systems found satisfactory in air at normal pressures.

There are a number of printing errors, most of which should be readily detected. More disconcerting is the omission at times of the unit of temperature, as both Fahrenheit and Celsius scales are used.

Lyons's book should be useful to workers in the plastics, textile, and user industries with an interest in flame retardancy and to those engaged in the production and testing of flame retardants.

G. W. V. STARK

Testis

The Testis. Edited by A. D. Johnson, W. R. Gomes and N. L. Vandemark. Vol. 1: Development, Anatomy and Physiology. Pp. xv+684. (Academic: New York and London, October 1970.) £18.20.

THE editors have assembled a team of contributors with widely different backgrounds and attitudes and the result is a volume which should find a similarly wide readership. The material is well chosen, profusely illustrated and supported by a massive bibliography.

"Development of the mammalian testis" by H. T. Gier and G. B. Marion has a welcome orientation towards the domestic species. The authors are well qualified to deal with the topic but occasionally they fail to make allowance for less specialized knowledge. I am still uncertain about the precise identity of the "weak triangle" referred to in the discussion of the descent of the testis in rodents. It is not a criticism of the chapter to say that the use of "cephalic ligament" for diaphragmatic ligament and of "gonadal ligament" for inguinal ligament are examples of the inconvenience of the lack of an agreed nomenclature. I found the line drawings excellent but I have doubts about the value of some of the photomicrographs, sometimes inadequately labelled.

Existing information about "The nerves of the testis, epididymis and scrotum" is effectively reviewed by Norma Hodson.

"Testicular blood supply, lymphatic

drainage and secretion of fluid" is an impressive contribution by B. P. Setchell. The review of the comparative anatomy of the blood supply and lymphatic drainage is extensive, magnificently illustrated and must become a standard reference source. The same high standard of scholarship is evident in the sections dealing with the physiology of blood and lymph flow in the testis and with the origin and physiology of the testicular fluids.

Because of his own contributions to research in this field G. M. H. Waites is well qualified to examine the mechanisms whereby the temperature of the testis is maintained below that of the body. "Temperature regulation and the testis" is a valuable personal account of present knowledge.

"Spermatogenesis" by M. Courot, Marie-Therese Hochereau-de Reviers, and R. Ortavant effectively covers the cytological and cytochemical events during spermatogenesis. The great value of this chapter is likely to be as a reference source for the advanced worker. Incidentally, although the authors have distinguished support for their view, I am still not convinced that the guinea-pig represents a particularly special case as regards the development of the acrosome. The editors have failed to remove some minor errors here: "Plasmatic membrane" (p. 362); "gonadic crests" (p. 364) presumably owe their appearance to imperfect translation. "Spermatoliosis" (p. 355) is presumably a printer's error.

"Sperm production rates" by R. P. Amann deals with the problems of measuring the production and output of spermatozoa by the testis and examines some of the results. The chapter illustrates admirably the community of interest of basic and applied science in this field.

"The testicular capsule" by J. R. Davis, G. A. Langford and P. J. Kirby fails to inspire confidence. The writing is never economical and often it is frankly tiresome. Doubts about the soundness of this chapter are not removed by statements such as "*in-vitro* undulating motions of the seminiferous tubule may be due to a type of Brownian movement". I found wholly unconvincing the arguments that the reduced size of the cryptorchid testis was due to the effects of changes in temperature on the contraction of the capsule and the conclusion that pharmacological studies of the capsule hold out promise of an effective male contraceptive.

"The intertubular tissue of the testis" by C. W. Hooker is essentially concerned with the morphology and physiology of the Leydig cells. This chapter is genuinely critical, it gives clear guidance about areas of uncertainty, it is comprehensive and yet

uncommonly readable. I would question, however, the interpretation of the observation that androgen production of the retained testis is reduced below normal levels in spontaneous cryptorchidism. This is presented as evidence that increased temperature impairs androgen production and this evidence is regarded as being opposed to evidence based on experimental cryptorchidism. Surely the situation in the natural cryptorchid is such that it is difficult to separate cause and effect.

The final chapter, "Fine structure of the testis and its functional significance" by M. H. Burgos, R. Vitale-Calpe and A. Aoki, is concerned much more with fine structure than with functional significance, but it combines a valuable text with a very beautiful atlas of the ultrastructural features of the cytological components of the testis. There are one or two minor distractions in the text such as the use of "human" as a noun and the remark (p. 561) that "figure 38 shows a very suggestive picture". And I found the description of the limiting membrane (p. 553) difficult to interpret; my difficulties were resolved when I realized that the sentence "the inner lamella contacts the cells of the tubule which in turn is made up of three layers" does not in fact mean what it says.

In conclusion I have to say that I know that I shall return to this book and I shall certainly direct others to it. It deserves the success which I am sure that it will have. J. C. HANCOCK

Drug Resistant Bacteria

Transferable Drug Resistance Factor R. Edited by Susumu Mitsuhashi. Pp. x+203. (University Park: Baltimore, London and Tokyo, December 1970.) \$14.50.

ONE of the most interesting bacteriological discoveries of recent years has been that of transferable drug resistance and Dr Mitsuhashi was prominent among those people who studied the phenomenon earliest and most intensively. A book from him is therefore of particular value. For several years, work on transferable drug resistance was confined to Japan, and many of the findings were published in Japanese journals, where they were not accessible to the large number of microbiologists of other nationalities who have since become interested. Mitsuhashi's book, which contains details of much of the early Japanese work, will be especially appreciated on this account. As well as editor, Mitsuhashi is author, or co-author, of every chapter of the book, giving it a unity of approach. The English may be faulty in places, but never to the point where it obscures the meaning.

R factors responsible for transferable drug resistance belong to the group of genetic elements known as bacterial plasmids which exist separately from the bacterial chromosome, but whose replication is more or less tightly coordinated with it, depending on the individual plasmid and on the species of host. "Transferable" is used here to mean transfer by conjugation, and transferability is conferred by a set of genes known as the sex factor of the same general nature as those in the *F* sex factor responsible for genetic recombination in *Escherichia coli* K12. Antibiotic resistance is conferred by genes which, in those cases where their action is understood, determine the production of enzymes destroying the activity of the antibiotic molecule.

The first chapter of Mitsuhashi's book is a history of the evolution of drug resistance amongst bacteria, chiefly *Shigellae*, in Japan and the emergence about 1957 of strains which had developed resistance to three or four drugs in a way that could not be reconciled with the process of repeated mutations and selection generally acceptable at the time. This introduction leads into an account of the discovery of *R* factors and of the prevalence and geographical distribution of different combinations of resistances, which is further developed in chapter 2. *R* factors can be transmitted to, and maintained by, many Gram-negative species, although with vastly differing efficiency.

A chapter with Dr Harada deals with the physiology of transfer, the structure of the *R* factor DNA molecule, control of its replication, and superinfection immunity. The chapter on genetics, with Dr Hashimoto, includes the resistance characters as well as those concerned with replication and transfer, and contains much detail of individual factors. The ability of *R* factors to coexist with *F* has made complementation studies possible, and the inability of two similar *R* factors to coexist has facilitated selection of recombinants. The other methods of genetic analysis described involve transduction by phage, analysis of plasmid segregation patterns, and recombination or complementation between segregants having point mutations in their resistance genes.

Dr Sawai is joint author of the chapter on biochemical mechanisms of the resistances conferred by *R* factors, which are remarkable in differing from drug-resistance acquired by chromosomal mutation. These mechanisms have done little, so far, to reveal the origin of *R* factors—the subject of the last chapter of the book. The most commonly found *R* factor-determined β -lactamase is similar in its properties to an enzyme often produced by

Klebsiella strains, and chloramphenicol acetylase like that determined by *R* factors has been found in various enterobacteria and in staphylococci. Nevertheless, these are no more than possible indications because there is, as yet, no firm evidence that the enzymes here are not still the products of plasmid genes. ELINOR MEYNELL

Reptiles Completed

Traité de Zoologie: Anatomie, Systématique, Biologie. Publié sous la direction de Pierre P. Grassé. Tome XIV: Reptiles—Glandes Endocrines—Embryologie—Systématique—Paléontologie (Fascicule III). Pp. 681–1428. (Masson: Paris, 1970.) 240 francs.

THIS volume completes the survey of reptiles in the *Traité*.

There are splendid chapters on the endocrine organs variously written by Saint-Girons, Gabe and Martoja. As a systematist I am tremendously grateful to such specialists, using the latest histochemical techniques, who have done so much more than obtain some specimens from a local dealer and then make pronouncements on reptiles. They have acquainted themselves with the problems of reptile systematics and have gone to much trouble to obtain a wide selection of material. At the end of a generously illustrated technical account they discuss the bearing which they consider their findings to have on reptilian systematics. The systematist who would use all available evidence has too often to wade through technical accounts of matters with which he has had no contact above undergraduate level, if that, and hope that he is extracting the significant information.

There are chapters by Guibé on the urinogenital system, reproduction, autotomy and regeneration, ecology and population dynamics. The reproduction and ecology chapters review much recent and interesting work and the survey of population dynamics is a welcome innovation. Matthey has a good review of reptilian chromosomes, an area which is now growing fast. Grassé considers the present state of knowledge of parthenogenesis in reptiles, a new and unexpected development. In an important chapter on development Pasteels draws together a wealth of scattered information. He gives an account of the descriptive and experimental analysis of early development as far as it is known and follows with an outline of organogeny. Pasteels generalizes as far as the fragmentary information permits and at the same time clearly indicates the major lacunae in our knowledge.

Unfortunately substandard is the chapter on geographical distribution,

which looks as though it was written in a hurry, without so much as a glance at Darlington, and contains a number of errors.

There are large sections on the systematics of Recent and fossil reptiles by Guibé and Ginsburg respectively. They survey the groups down to family level. In the Recent reptile chapter much new information has been incorporated into the characterization of the higher taxa; I found very few errors. The fossil reptile chapter has an extensive discussion of the phylogeny of reptiles; the author rejects the major division into Sauropsida and Therapsida and adduces support for the recent suggestion of Reig that the Archosaurs originated from Pelycosaurs. There are some interesting innovations, with supporting arguments, in the placement of some forms, such as *Bolosaurus* and *Trilophosaurus*.

There are fewer cases than in the first "fascicule" of the same species occurring under different names but some of the literature citations are deplorable. In one chapter I counted 190 references to the work of other authors (some several times). For 108 of these I either could not find in the literature list a publication of the cited date or, in the case of 62 authors, any mention of the name at all! The contributors to and the purchasers of such an expensive and well turned out volume deserve better than this.

GARTH UNDERWOOD

Electron Microscopy

Principles and Techniques of Electron Microscopy: Biological Applications, Vol. 1. By M. Arif Hayat. Pp. xiv + 412. (Van Nostrand Reinhold: New York and London, February 1971.) £9.75.

In the preface the author states that "... the primary objective of this book is to provide the reader with the foundation in biochemical concepts governing the preparatory procedures". Essentially he has succeeded in doing this, and has provided, at the same time, a very readable volume covering the important practical techniques of fixation, embedding, sectioning, staining, and supporting of sections for examination under the electron microscope. The integration of basic chemical considerations and practical details of technique is particularly satisfactory: it is unfortunate, however, that some of the structural formulae, particularly in the section on embedding, are inaccurate or incomplete.

The problem of where, and how, osmium is deposited in biological tissues, central to the interpretation of the "unit membrane" structure, is fully covered in the chapter on fixation. There is, however, some repetition of

the data on the oxidation of double bonds by OsO_4 on pages 40 and 41. The proposed migration of reduced osmium from the apolar layer to the polar groups of the phospholipid is dealt with in a less than precise manner; it is suggested that the osmic acid monoester group (shown as covalently bound to the lipid alkyl chain) migrates by virtue of an electrostatic interaction with the polar head group. Presumably even this would not be possible if the very stable diester were to be formed, this reaction being put forward as the explanation for lipid fixation by osmium tetroxide. The author rightly comments that further information is needed to elucidate the role of the lipid polar groups in the fixation of biological tissue by osmium tetroxide.

The chapters on fixation, embedding, and sectioning contain a considerable amount of practical information, and the one on sectioning is particularly good: this chapter does, however, contain the extraordinary statement that sodium hydroxide is entirely volatile! A table of defects appearing during sectioning is included, and this consolidates much of the information contained in the chapter, as a practically useful "trouble-shooting" guide.

The presentation is generally good, but it is unfortunate that so many typographical errors remain in the text: it seems that text has been omitted on pages 59 and 60, and that the legend to Figure 1-8 is incomplete.

This book may be recommended for graduate students, and for more experienced research workers who may wish either to become acquainted with the general preparative procedures associated with electron microscopy, or to have access to a compendium of practical techniques for use in the laboratory. There is a comprehensive bibliography and author index, as well as an appendix with information on the making-up of fixing and buffer solutions, together with details of certain special fixing procedures. The list of reagent suppliers should be of special use to those workers in the United States, rounding off as it does a clear and comprehensive volume on this aspect of electron microscopy.

R. A. KLEIN

Metallurgy Writ Large

Physical Metallurgy. Edited by R. W. Cahn. Second revised edition. Pp. xxi + 1333. (North-Holland: Amsterdam and London; Elsevier: New York, 1970.) £18.65.

THE first edition of this monumental work was a success. The appearance of a second edition so soon afterwards is no reflexion on the quality of the first, but, rather, is indicative of the rapid

development of the subject. Practical metallurgy has evolved over many centuries more as an art than a science whereas physical metallurgy, the basic science of metallurgy, has developed in the past fifty years or so in parallel with modern techniques of structural examination of solid materials—X-ray and electron diffraction, electron microscopy, electron-probe analysis, and the like.

The subject is now presented, with extensive revision and an additional chapter on the physical metallurgy of steels, in a logical and authoritative manner in twenty-three chapters by different authors. In my opinion, two topics are still inadequately covered—first, deformation and re-crystallization textures and associated anisotropy and, second, structure and properties of the metallic surface.

The text is already extensive and the volume is beyond the pocket of the average student. The work, however, has established itself as the standard text for all technical libraries. It is hoped that the authors will continue to revise and bring their contributions up to date.

Although it is essential that there should be a full and authoritative text on physical metallurgy, one cannot help feeling that the practical metallurgist has been left far behind. There is clearly a need for a further text on applied physical metallurgy in which the basic principles so well propounded in the full text are directly related to the practical problems of metal manufacture and usage.

T. LL. RICHARDS

Microbial Corrosion

Microbial Aspects of Metallurgy. Edited by J. D. A. Miller. Pp. 202. (Medical and Technical Publishing: Aylesbury, 1971.) £3.40.

THE ever-increasing interest in, and importance of, environmental and interdisciplinary science has created a need for a book of this nature and on this subject. It is a disappointment, therefore, that a better job has not been made of it. This is not to decry the amount of information packed into this small book but rather the method and style of presentation. The book is based on a short series of lectures given at the University of Manchester Institute of Science and Technology and has retained the digressions and amicable dalliances on matters of small relevance that enliven a short course, but that only serve to irritate when translated into print.

The first chapter on introductory microbiology is specially prone to these faults, attempting to cover too wide a

spectrum too briefly, instead of concentrating on matters wholly relevant to the rest of the book. The second chapter on introductory corrosion firmly delineates at the outset those aspects of a vast subject on which it is proposed to concentrate. The result is an excellent digest that is readily understandable to a newcomer to the subject and contains all that is required to pursue the remaining sections of the book.

The four chapters which follow deal in detail with various aspects of microbiological corrosion and comprise an informative and mostly highly readable account of a very wide variety of problems from both theoretical and practical considerations. A quiet word, however, should have prompted the author who coined the appalling term "desulfovibriologist" to think again!

The section entitled "Microbial Corrosion of Buried and Immersed Metal" concentrates rather excessively on the work of one particular school of investigators and might well have been placed after the succeeding chapter on corrosion in industrial situations of which it forms but one, even if probably the most important, example.

The chapter on microbial corrosion in aircraft fuel systems is particularly well ordered and concentrated and that on microbial infections in relation to corrosion ends with a practical appendix that should prove very useful to a novice wishing to embark on a little practical investigation into his own problems.

The book ends with two short chapters on the more constructive activities of microorganisms in aiding the extraction of metals from low-grade ores. The first of these, very short, is almost wholly redundant, there being little in it of relevance that is not repeated more clearly and concisely in the second.

G. H. BOOTH

Microscopes Confined

Some Biological Techniques in Electron Microscopy. Edited by D. F. Parsons. Pp. x+186. (Academic: New York and London, August 1970.) £4.65.

BIOLOGICAL electron microscopy has made great progress in the twenty years since the development of thin sectioning techniques. Histology and many aspects of physiology have had to be rewritten, yet there are still technical limitations that impede the realization of the full potentialities of electron microscopy. This book indicates some of the problems. In the longest chapter, Parsons discusses the physical factors that limit the resolution of biological materials. He deals with aberrations, specimen damage and specimen contrast. The biologist tends to ignore the effects of thermal and radiation damage but it is a depressing

fact that electron diffraction patterns from most biological crystals disappear within a few seconds. Reduction of specimen damage may yet turn out to be the chief advantage of high voltage electron microscopy. The chapter ends with an interesting discussion of the possibilities of electron phase contrast and related methods.

The next two contributions, on chemical effects of fixation and on freezing techniques by Riemersma and Bullivant respectively, deal with topics of immediate practical importance. Unfortunately, the article on fixation is mainly restricted to osmium tetroxide and potassium permanganate; the aldehydes are mentioned almost as an afterthought. Freezing methods, apart from freeze-etching and freeze-fracturing, have been rather disappointing so far, but progress will have to be made if we are ever to use electron microscopy to locate diffusible ions and molecules.

The fourth main article is on substrate noise, by Harris. It deals with the effects of the supporting medium, beam potential, the ideal substrate and optical methods for improving the signal/noise ratio. Optical image processing is an expanding field and may have an important part to play in electron microscopy. Finally, there is a short account by Banfield of attempts to introduce automation into tissue processing for electron microscopy.

This book, like others of similar type, suffers from some lack of coherence and from variations in quality and approach. Nevertheless it contains much of interest and many useful references for those who want to keep in touch with current ideas and future possibilities.

R. BARER

Orbits Transformed

Linear and Regular Celestial Mechanics: Perturbed Two-body Motion, Numerical Methods, Canonical Theory. By E. L. Stiefel and G. Scheifele. Pp. ix+301. (Springer-Verlag: Berlin and New York, 1971.) 68 DM; \$18.70.

THIS book is concerned with ways of formulating the differential equations that occur in celestial mechanics so that they become both linear and regular. The usual equations of motion for the two-body problem have a singularity corresponding to the collision of the two bodies. By a suitable transformation of variables, the equations can be regularized so that this singularity disappears. The resulting equations are also linear. These regularized equations have been used by many authors to study collision and near-collision orbits. The emphasis in this book is more on the advantages to be obtained from the linearity.

Before 1965, the only regularizing transformations known were for two-dimensional motion, which rather res-

tricted the application of the technique to problems of a theoretical nature. The regularization of the differential equations of three-dimensional motion was achieved by Kustaanheimo and Stiefel in 1965, and the book is essentially a discussion of what is known as the KS-transformation.

The theory of the transformation is first described. The equations of motion are derived in terms of the transformed coordinates and also in terms of a set of elements analogous to the orbital elements of the classical theory. A useful feature of this part of the book which makes the mathematics easier to follow is the collection at the end of each chapter of the important equations derived therein.

The transformed equations are simpler than the classical, but the number of equations is increased. The authors show by numerical examples that, as a result, the two sets of equations can be integrated numerically on a computer in approximately equal times. They also show that the regularized equations are more stable than the classical equations so that the errors in the numerical integration build up more slowly.

The transformed equations of motion are derived for a body perturbed by a third body and by the oblateness of its primary, and examples of the numerical integration of these equations are given. This should make it possible for anyone faced with a practical problem of numerical integration in celestial mechanics to use these methods without spending too much time understanding the theory. The transformed equations for unperturbed two-body motion are the equations of a harmonic oscillator, and the authors develop several numerical methods to integrate these equations exactly. They show by numerical examples that these methods are more accurate for perturbed motion than ordinary methods.

The second part of the book is concerned with canonical theory. First, there is a derivation of the classical equations of motion in canonical form, and then various canonical forms of the transformed equations are derived, one of them having the eccentric anomaly as the independent variable. This is particularly advantageous as it is shown that Fourier expansions of the perturbation function in terms of the eccentric anomaly generally converge better than those in terms of the mean anomaly which are usually used in celestial mechanics.

In the third part of the book, various geometric and topological properties of the KS-transformation are discussed. The authors conclude that the search for other transformations to regularize the attraction of a single centre is not very promising. The KS-transformation promises to play an important part in future developments in celestial mechanics. This book is a clear exposition of it.

A. T. SINCLAIR

CORRESPONDENCE

Human Embryology

SIR,—Edwards and Sharpe (*Nature*, 231, 87; 1971) assert that "The common law of early centuries dealt only with either the 'mother or fully delivered child'," citing Coke thereto.

I know of no better authority than Coke as to the general tenor of the law save the written law itself, which, standing "since the mind of man runneth not to the contrary", is assimilated to the common law, and is explicit; Alf. c 58 (sometime numbered c 9); "Be tham thæt man ofslea wif mid cilde: Yif mon wif mid bearne ofslea thonne thæt bearn in hire sie, foryielde thone wifman fullan yelde, & thæt bearn be thæs fædrencnosles were healfan yelde", which I transcribe as "By them that wrongfully strike wife with child: If anyone cast down wife with bairn when that bairn be in her, then yield for the woman full compensation, and for that bairn half the compensation due in respect of the father's kin".

Yours faithfully,

TOM POPLETT

3 Bernhard Baron Cottages,
Polegate,
Sussex

Smoke Without Fire

SIR,—Your article "Smoke Without Fire" (*Nature*, 230, 418; 1971) contained a useful analysis of the current situation relative to a Comprehensive Nuclear Test Ban Treaty. The opportunities for achieving such a treaty appear better now than at any time since 1963, and it would serve as a useful complement to a SALT agreement in limiting nuclear arsenals.

However, your article was seriously marred by the concluding sentence which stated that such a treaty would do away with the need for IAEA inspection to verify compliance with the Non-Proliferation Treaty. Unfortunately this is not so. A non-nuclear weapons nation that had unsafeguarded fissionable material could proceed with a nuclear weapons programme without carrying out any nuclear tests. A simple atomic bomb could be developed

today without any nuclear explosions, provided fissionable material was available. The basic weapons design principles are well known, and most of the detailed engineering can be confirmed without an actual nuclear test. No nation has yet had a failure in its first atomic test.

Therefore, IAEA safeguards on fissionable material produced in peaceful programmes are essential if proliferation is to be controlled. There is no basis for saying that these are an international hazard in their own right. They are being developed by the IAEA in a manner to avoid compromise of industrial secrets, and the US and the UK governments have voluntarily agreed to accept them even though not required to do so by the NPT.

Yours faithfully,

HERBERT SCOVILLE JUN.

6400 Georgetown Pike,
McLean, Virginia 22101

Graduate Footballers

SIR,—For a regular, albeit non-scientific, reader to find an error in *Nature* is almost as profound a shock as if the mistake had been committed by nature herself. Yet there they are—not one error, but two, and in the same sentence in the same leader: "No Dole at the Top" (*Nature*, 231, 70; 1971).

To call that astonishing professional newcomer Michael when everyone within 10 miles of north London let alone Liverpool must know he is Mr Steve Heighway is bad enough, but then you add insult to the injured sensibilities of all football addicts by proclaiming him "the only professional footballer in Britain to have been to university and to have come away with a degree". Oh, sir, and it is his very own teammate, Mr Brian Hall, who gives you the lie.

Yours faithfully,

RICHARD B. FISHER

26 St Paul's Road,
London N1

Labour Rewarded

SIR,—Why should it be thought natural¹ to assume that an animal will prefer to obtain food without work? To me it seems much more reasonable to suppose that the work which normally accompanies an animal's search for food should be enjoyable to the animal. It may at times even be performed with the full knowledge that it will not lead to food. For surely enjoyment of an animal's necessary functions has a high survival value.

With dogs growling naturally goes with the taking of food; it is, in the sense in which the word is used here, work. It is obvious that dogs enjoy it and indulge in it even when food is not expected. My dog will often, though apparently hungry, refuse to touch his food until I have made a symbolic gesture of taking it away from him, whereat he will growl and fall to. His wagging tail proclaims his enjoyment.

Yours faithfully,

H. H. CLAYTON

75 Glendale Avenue,
Deep River,
Ontario

¹ *Nature*, 229, 89 (1971).

Our experimental psychology correspondent comments:

One of the principles that has been used, explicitly or implicitly, in explaining how animals come to learn certain tasks is that they attempt to minimize the effort expended. So although the point that animals are adapted to working for their food is certainly a reasonable one, there remains the question as to why an animal chooses the shorter of two routes through a maze, or comes to prefer the less onerous of two reinforcement schedules. The assumption that he is minimizing effort may of course be quite wrong; an alternative index that the animal might use is the time between starting a trial and achieving reward. It is in any case an empirical question, and the recent experiments on preferences for working over receiving free food demonstrate that there is a problem. Quite apart from any theoretical issue that might be involved, I think that few people would predict that an animal or person would invariably choose the more laborious of two alternative means of achieving a goal.

Announcements

University News

Dr T. E. Allibone, who recently retired as chief scientist at the Central Electricity Generating Board, has been appointed visiting professor in the Department of Physics, **City University**.

Dr Ivor Evans, deputy director of the Mining Research and Development Establishment, has been appointed special professor in mining engineering at the **University of Nottingham**.

Dr R. S. Asquith has been appointed to the chair of industrial chemistry at the **Queen's University of Belfast**, and **Dr P. F. D'Arcy** has been appointed to the chair of pharmacy.

The title of professor of structural mechanics in the Department of Civil Engineering has been conferred on **Mr H. Tottenham**, reader in that department, **University of Southampton**.

Miscellaneous

His Majesty Emperor Hirohito of Japan has been elected a fellow of the **Royal Society**.

The Lampitt medal of the **Society of Chemical Industry** has been awarded to **Mr Horace Hayhurst**, former chairman of the Manchester Section and the Pesticides Group.

Dr J. G. Charney, professor of meteorology at the Massachusetts Institute of Technology, has been awarded the **International Meteorological Organization prize**.

Professor L. J. Bruce-Chwatt, London School of Hygiene and Tropical Medicine, and **Professor A. Corradetti**, Istituto Superiore di Sanita, Rome, have been awarded the **Darling prize of the World Health Organization**, for outstanding work in the fight against malaria.

Notes to Authors

Will contributors to *Nature* follow as far as possible the general style of the journal, and in particular the following rules :

1. Manuscripts should be typed in double spacing and submitted in duplicate. Two copies of all figures should be included, one for the printers and one for the referee.

2. References are indicated by superscript numbers in the order in which they appear in the text, and have the following form :

¹ Sargent, W. L. W., and Searle, L., *Astrophys. J. Lett.*, **162**, L155 (1970).

² Fraenkel-Conrat, H., in *Comprehensive Biochemistry* (edit. by Florkin, M., and Stotz, E. H.), **7**, 75 (Elsevier, Amsterdam, 1963).

"Unpublished work", "Private communication" and "Work in preparation" should not be cited as references but may be incorporated in the text. Each reference number should refer to an individual work which has either been published or is in the press.

3. Although the text of longer articles is usually interrupted by headings at suitable intervals, these should be short and informative and not merely "Introduction", "Results", or "Conclusion".

4. Units should either conform to the SI system or be spelled out in full.

A fuller guide can be had from the *Nature* offices in London and Washington.

Wellcome Trust Animal Health fellowships have been awarded to **Mr D. F. MacDougall**, to study immunoglobulin metabolism in young calves, at the University of Glasgow, and to **Mrs R. M. Aitken**, to study the immune response in the fowl, at the Royal (Dick) School of Veterinary Sciences.

International Meetings

June 8-9, **Using Natural Resources in Work and Leisure**, London (Secretary, Institute of Rural Life at Home and Overseas, 27 Northumberland Road, New Barnet, Hertfordshire).

June 14-17, **Identification and Measurement of Environmental Pollutants**, Ottawa (Mr M. K. Ward, Executive Secretary, ISIMEP, c/o National Research Council of Canada, Ottawa 7, Ontario, Canada).

June 26-27, **World Federation of Nuclear Medicine and Biology Assembly**, Los Angeles (Society of Nuclear Medicine, 211 East 43rd Street, New York, New York 10017, USA).

British Diary

Tuesday, June 8

Molecular Biology of Synaptic Receptors (6.30 p.m.) Professor E. de Robertis, Biochemical Society, Neurochemical Group, at Charing Cross Hospital Medical School, Chandos Place, London WC2.

Aerospace Antennas (three-day conference) Institution of Electrical Engineers, in association with the Institution of Electronic and Radio Engineers, at the Institution of Electrical Engineers, Savoy Place, London WC2.

Wednesday, June 9

Contemporary Methods for the Analysis of Metals (symposium) Dr J. A. W. Dalziel, Professor T. S. West, Mr R. H. Reynolds and Mr N. T. Gallagher, Polytechnic of the South Bank, Borough Road, London SE1.

Teaching of Instrumentation in Engineering Courses (two-day conference) Institution of Mechanical Engineers, at Brunel University, London.

HOW TO BUY NATURE

Volumes start in January, March, May, July, September and November, but subscriptions may begin at any time.

The direct postal price per subscription is:

12 MONTHS* (52 issues per title)

	Surface Mail UK and worldwide	Airfreight U.S.A.	Canada
Nature (Friday)	£14	\$48	\$52
Nature + Nature Physical Science	£24	\$83	\$90
Nature + Nature New Biology	£24	\$83	\$90
All three editions	£29.50	\$108	\$116
Annual Index	£1	\$3	\$3

* Rates for shorter periods *pro rata* (minimum three months)
(Charge for delivery by air mail on application)

Editorial and Publishing Offices of NATURE

MACMILLAN JOURNALS LIMITED
4 LITTLE ESSEX STREET, LONDON WC2R 3LF
Telephone Number : 01-836 6633. Telegrams : Phusis London WC2R 3LF

711 NATIONAL PRESS BUILDING
WASHINGTON DC 20004
Telephone Number : 202-737 2355

Subscription Department

MACMILLAN JOURNALS LIMITED
BRUNEL ROAD, BASINGSTOKE, HANTS
Telephone Number : Basingstoke 5431

Advertisements only should be addressed to

T. G. SCOTT & SON, LIMITED
1 CLEMENT'S INN, LONDON WC2A 2ED
Telephone : 01-242 6264/01-405 4743
Telegrams : Textualist London WC2A 2ED

Registered as a newspaper at the Post Office

Copyright © Macmillan Journals Limited, June 4, 1971